VOL. XXII. [JULY, 1900.] NO. 7.

THE JOURNAL

OF THE

AMERICAN CHEMICAL SOCIETY.

[CONTRIBUTIONS FROM THE SHEFFIELD LABORATORY OF YALE UNIVERSITY.]

ON UREAIMIDO ESTERS, THIOUREAIMIDO ESTERS, ACYLTHIOUREAIMIDO ESTERS, AND UREAAMIDINES.

[PRELIMINARY PAPER.]

BY HENRY L. WHEELER AND W. MURRAY SANDERS.

REPRESENTATIVES of new classes of compounds, isomeric or metameric with the acylpseudourea ethers, acylpseudothiourea ethers and diacylpseudothiourea ethers, can be prepared by combining isocyanates, mustard oils, and acyl rhodanides with imido esters. The imido esters immediately combine with phenyl isocyanate, at ordinary temperatures, giving a quantitative yield of an addition product as follows:

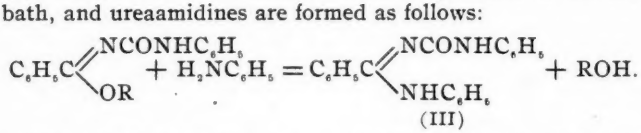
$$C_6H_5C\begin{matrix}\diagup NH\\ \diagdown OR\end{matrix} + CONC_6H_5 = C_6H_5C\begin{matrix}\diagup NCONHC_6H_5\\ \diagdown OR\end{matrix}$$

This class of compounds, perhaps, may be called ureaimido esters or carbamidimido esters, since they have the structure of imido esters and ureas combined. They are extremely sensitive towards hydrogen chloride by which reagent, just as the acylpseudourea ethers[1] (II) they are readily and quantitatively converted in the cold, with loss of alkyl chloride, into acylureas.

[1] Stieglitz and Dains: This Journal, **21**, 136.

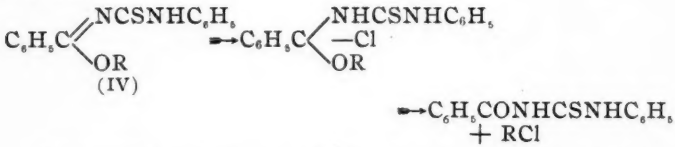
This reaction with these two classes of compounds proceeds as follows:

It will be noticed that the imido ester grouping in the different positions in these isomers are affected alike, inasmuch as under similar conditions both compounds give with hydrogen chloride the same derivative, benzoylphenylurea. The ureaimido esters (I) readily react with aniline, at the temperature of the steam-bath, and ureaamidines are formed as follows:



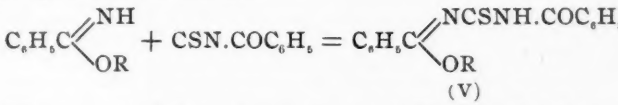
Whether the structure shown in formula (III) or the tautomeric form is to be assigned to these amidines must be decided later. These amidines have little or no basic properties.

The mustard oils, phenylmustard oil and allyl mustard oil, react less energetically than phenyl isocyanate with the imido esters. On long standing or on warming, the mixtures deposit crystals. These compounds, for which the name thioureaimido esters (IV) is proposed, are closely similar to the above oxygen analogues, since with hydrogen chloride, under similar conditions, they readily separate alkyl chloride and give the corresponding acylthio ureas. The mechanism of the reaction is as follows:



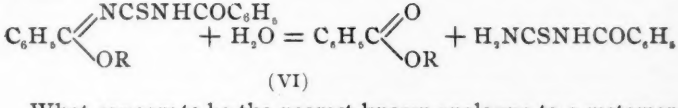
The isomeric acylpseudothiourea ethers and the diacylpseudothiourea ethers apparently have not yet been prepared, so that a comparison of the behavior of these compounds with the thioureaimido ester derivatives must be deferred to a later date.

When the thioureaimido esters are warmed with aniline, and other organic bases, thioureaimidines are formed. Like the above ureaamidines they appear to have no basic properties. The fact that they are not soluble in alkali affords new evidence that the double union of the imido ester grouping confers a basic nature to the molecules. Here, however, it is neutralized by the negative character of the urea group. The imido esters combine with acetyl and benzoyl thiocyanates with evolution of heat, the mixtures readily solidifying. Acylthioureaimido esters (V) are then produced, as follows:



(V)

These compounds are formed more readily than the above and they are far more unstable than the thioureaimido esters. When they are warmed with hydrochloric acid they decompose in a different manner from the other types of ureaimido esters. A monoacylthiourea, and not a diacyl derivative, is obtained. The decomposition under this treatment takes place at the double union as follows:



(VI)

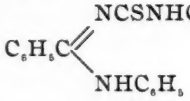
What appears to be the nearest known analogue to a metamer of the above or dibenzoylpseudothiourea ether is the ethyliso-dicarbethoxyurea, $C_2H_5O.CON=C\begin{matrix}NCO.OC_2H_5\\OC_2H_5\end{matrix}$, of Dains.[1] This compound is also very sensitive towards hydrogen chloride; however, it does not separate at the double union, like the above, but decomposes with the evolution of ethyl chloride, giving the diacylurea, $C_2H_5OCONHCONHCO.OC_2H_5$. The ease with which the ureaimido esters decompose with hydrochloric acid, without forming a stable intermediate addition product or "salt," recalls the perfectly analogous behavior of the acylimido esters.[2] In the case of the latter, the above two reactions with

[1] *Loc. cit.*

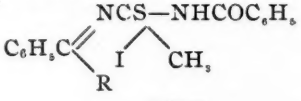
[2] Wheeler, Walden and Metcalf: *Am. Chem. J.*, **19**, 129 and **20**, 64.

hydrogen chloride were also observed. The properties of the ureaimido esters, in general, approach more nearly to those of the acylimidoesters than to those of other types of imido esters.

When the acylthioureaimido esters are treated with aniline, toluidine, etc., acylthioureaamidines (VII) are formed in the normal manner. These compounds, as well as the thioureaimido esters, combine with alkyl halides, the addition probably taking place to the sulphur as shown in formula (VIII).



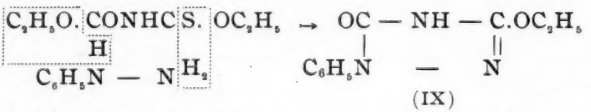
(VII)



(VIII)

The action of phenylhydrazine on the acylthioureaimido esters seemed theoretically promising for the preparation of triazole derivatives; but it appears, from preliminary experiments, that, although hydrogen sulphide is evolved in this treatment, triazoles do not result, at least not as the chief product. The hydrogen sulphide, first formed, decomposes a part of the ureaimido ester, giving benzoylthiourea, etc.

Experiments in this laboratory by Dr. Bayard Barnes have shown that the acylphenylthioureas react with aniline and phenylhydrazine with the removal of the acyl group. With phenylhydrazine no triazole derivative was obtained. Similar results were obtained with the acylphenylurethanes, acetylphenylurethane, formylphenylurethane[1] and acetylphenylthiourethane. It is probable that other diacylanilides will be found to act similarly. On the other hand those acylurethanes which do not contain a phenyl group such as acetylurethane,[2] acetyl- and benzoylthionurethane, give triazole derivatives. Dr. Barnes has also found that carbethoxythionurethane of Delitsch[3] readily reacts with phenylhydrazine as follows:

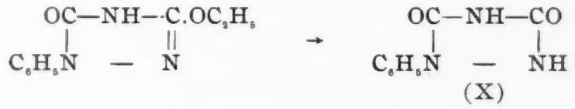


(IX)

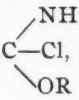
That the compound formed in this reaction is 1-phenyl-3-

[1] Wheeler and Metcalf: *Am. Chem. J.*, **19**, 217.
[2] Andreocci: *Ber. d. chem. Ges.*, **22**, R. 737; *Ibid.*, **24**, R., 203.
[3] *J. prakt. Chem.*, **10**, 118.

ethoxy-5-triazolon, and that it has the structure represented by formula (IX), is shown by its behavior, on boiling with strong hydrochloric acid, whereupon it is converted into Pinner's[1] urazole, (X):

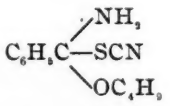


(X)

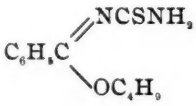
The process which in general serves for the preparation of simple or monosubstituted ureas and thioureas, and which consists of treating a salt of an amine with potassium cyanate or thiocyanate, does not lead to the formation of the prototypes of the above urea derivatives, when applied to the salts of the imido esters. This fact seems to the writer to be a strong argument in support of the theory of Stieglitz that the salts of the imido esters are not ammonium derivatives, but that they are derived from the form  , etc. In fact, the properties of the cyanic and thiocyanic acid salts do not agree with those which would be expected for salts having an ammonium structure.

When ice-cold solutions of benzimidoisobutyl ester hydrochloride and potassium cyanate, in molecular proportions, are mixed together, a mass of well-crystallized, colorless prisms separate. If an attempt is made to filter these at ordinary temperature they rapidly decompose, giving off a gas (CO_2?), and completely liquefying. Owing to the unstable nature of this salt, which is undoubtedly a cyanate, it was not examined further. On the other hand, the corresponding thiocyanic salt is far more stable. It can be prepared, at ordinary temperature, by mixing solutions of the imido ester hydrochloride with potassium thiocyanate, whereupon a bulky mass of colorless prisms or needles separate. This salt has the structure represented by formula (XI), and all attempts to rearrange it into the metameric thioureaimido ester (XII) failed.

[1] *Ber. d. chem. Ges.*, **21**, 1219.

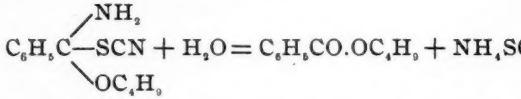


(XI)

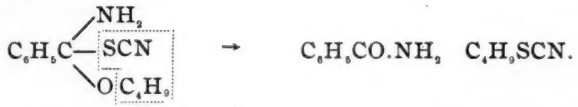


(XII)

When this salt is warmed in aqueous solution it dissolves, but the solution almost immediately becomes turbid and an oil (isobutyl benzoate) separates. On evaporating to dryness nothing but ammonium thiocyanate is obtained. The decomposition then proceeds as follows:

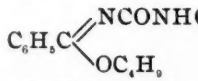


If the dry salt is heated above its melting-point it decomposes smoothly as follows:



It will be noticed that these reactions are analogous in every respect to those of the hydrochloric acid salts of the imido esters.

EXPERIMENTAL PART.

Phenylureaimidoisobutyl Benzoate,  .—Phenyl isocyanate (3.4 grams) and benzimidoisobutyl ester (10 grams) combined with evolution of heat and the mixture solidified to a white mass. When this was crystallized from alcohol, colorless prisms were obtained which melted from 99°–100°. A nitrogen determination gave:

	Calculated for $C_{18}H_{20}N_2O_2$.	Found.
Nitrogen.....	9.4	9.1

Action with Hydrogen Chloride.—Some of the above material was dissolved in benzene and the solution was saturated with hydrogen chloride whereupon colorless prisms separated, melting at about 200°. This material proved to be *Benzoylphenylurea*, $C_6H_5CONHCONHC_6H_5$, which crystallized from alcohol in long

silky needles, and on boiling it with sodium hydroxide it gave phenylurea.

The extreme sensitiveness of compounds of this type towards hydrochloric acid was shown by the fact that it was found to be impossible to isolate ureaimido esters from benzimidomethyl, ethyl, and phenylacetimidomethyl esters and phenyl isocyanate, if the latter contained phosphorus oxychloride. Under these conditions the benzimido esters gave *benzoylphenylurea*, while phenylacetimidomethyl ester gave a compound very difficultly soluble in alcohol. It crystallized in the form of needles and melted at 166° and a nitrogen determination showed that it was *phenylacetylphenylurea*, $C_6H_5CH_2CONHCONHC_6H_5$:

	Calculated for $C_{15}H_{14}N_2O_2$.	Found.
Nitrogen	11.0	11.0

Action with Aniline.—Phenylureaimidoisobutyl benzoate (2 grams) and aniline (0.6 gram), were mixed in benzene and warmed on the water-bath whereupon a substance separated in granular crystals. It was found to be very difficultly soluble in alcohol (a property of ureaamidines, which is general as far as we have observed, and on boiling with alcohol it melted from 179°–180°. A nitrogen determination agreed with the figures calculated for *benzenylphenylureaphenylamidine*, $C_6H_5NH—(C_6H_5)C = NCONHC_6H_5$.

	Calculated for $C_{20}H_{17}N_3O$.	Found.
Nitrogen	13.3	13.0

This amidine is insoluble in dilute hydrochloric acid and no hydrochloric acid salt was obtained when the material was dissolved in benzene and saturated with hydrogen chloride. It is insoluble in alkali.

Phenylthioureaimidomethyl Benzoate, $C_6H_5C\begin{matrix}=NCSNHC_6H_5\\ \diagdown OCH_3\end{matrix}$.—Equal quantities of benzimidomethyl ester and phenyl mustard oil (5 grams of each) were mixed and kept at a temperature of about 60°–70° for two days. On cooling, then, the mixture solidified almost completely. In each of the experiments with phenyl mustard oil and the imido esters, a yellow mixture resulted although the ingredients before mixing were colorless.

On crystallizing from alcohol beautiful colorless or cream-colored lozenge-shaped tables were obtained which melted sharply at 132°. The material is readily soluble in benzene and hot alcohol, difficultly in cold alcohol, and a nitrogen determination gave:

	Calculated for $C_{15}H_{14}N_2OS$.	Found.
Nitrogen	10.37	10.29

When this compound was treated with cold dilute hydrochloric acid, in which it is insoluble, little or no action was observed after two days' standing. When a little alcohol was added and the whole was warmed, effervescence took place, methyl chloride was evolved, and, on cooling, the long characteristic colorless needles of *benzoylphenylthiourea*, $C_6H_5CONHCSNHC_6H_5$, were obtained, melting about 148°.

Benzenylphenylthiourea-p-anisylamidine, $C_6H_5C(=NCSNHC_6H_5)NHC_6H_4OCH_3$.—Molecular quantities of the above imido ester and *p*-anisidine were mixed in benzene solution and allowed to stand at a temperature of about 70° for several hours, whereupon a mass of plates or scales separated. This material was very difficultly soluble in alcohol and in benzene. When crystallized from these solvents and from amyl acetate, it melted at about 180°. A satisfactory nitrogen determination was not obtained, undoubtedly owing to the fact that this substance, as well as the corresponding phenyl and naphthyl amidines, burn with great difficulty, the results invariably coming low.

Phenylthioureaimidoethyl Benzoate, $C_6H_5C(=NCSNHC_6H_5)OC_2H_5$.—Benzimidoethylester (5 grams) and phenyl mustard oil (4.5 grams) gave a yellow oil directly on mixing. After two days the material solidified to a glistening mass of flattened prisms or tables and on crystallizing from alcohol it separated in the form of cream-colored tables and it melted sharply at 119°. These thioureaimido esters, in general, show a great tendency to crystallize. A nitrogen determination gave:

	Calculated for $C_{16}H_{16}N_2OS$.	Found.
Nitrogen	9.8	9.7

When treated with hydrochloric acid it behaved precisely like the methyl ester and benzoylphenylthiourea was obtained.

Phenylthioureaisobutyl Benzoate, $C_6H_5C\begin{matrix}=NCSNHC_6H_5\\ \diagdown OC_4H_9\end{matrix}$.—Benzimidoisobutyl ester (5 grams) and phenyl mustard oil (3.8 grams) behaved, on mixing and standing, precisely like the above. When crystallized from alcohol it forms large, stout tables and it melts at 125°. A nitrogen determination gave:

	Calculated for $C_{18}H_{20}N_2OS$.	Found.
Nitrogen	8.9	8.9

When this was treated with hydrochloric acid and warmed, *benzoylphenylthiourea* was obtained.

When 2 grams of this imido ester were warmed on the water-bath with aqueous-alcoholic ammonia for a number of hours and then allowed to cool, colorless crystals separated melting from 151°–153°. A nitrogen determination gave 18.5 per cent., while the calculated for benzenylphenylthiourea amidine is 27.0 per cent., and that for phenylthiourea which melts at 154° is 18.4 per cent. The material, in fact, had the bitter taste and other properties of *phenylthiourea*.

When this thioureaimido ester was treated with methyl iodide a varnish was obtained which crystallized after long standing.

Phenylthioureaisobutyl benzoate readily reacts with phenyl hydrazine, giving off hydrogen sulphide and it gives a compound which crystallizes in plates when treated with benzoyl chloride. These reactions will receive more thorough attention later.

Benzenylphenylthioureaphenylamidine, $C_6H_5C\begin{matrix}=NCSNHC_6H_5\\ \diagdown NHC_6H_5\end{matrix}$.—The above imido ester (4 grams) and aniline (1.1 grams) after warming on the water-bath gave off only a trace of hydrogen sulphide. The product, on crystallizing from alcohol, formed a beautiful mass of large thin plates. They melted from 151°–152° and a nitrogen determination gave:

	Calculated for $C_{20}H_{17}N_3S$.	Found.
Nitrogen	12.6	12.0

This material is insoluble in cold hydrochloric acid, concen-

trated or dilute. On warming, it dissolves, but on cooling it crystallizes out again unaltered. Platinum chloride precipitates a curdy yellow mass from the concentrated hydrochloric acid solution.

Benzenylphenylthiourea-β-naphthylamidine, $C_6H_5C\begin{matrix}=NCSNHC_6H_5\\ \diagdown NHC_{10}H_7\end{matrix}$.—The above imido ester readily reacts with β-naphthylamine, giving a compound that crystallizes in minute plates and which is extremely insoluble in the ordinary organic solvents; when crystallized from alcohol and amyl acetate it melted, not sharply, from 182°–183°. The material burned very slowly and a satisfactory nitrogen determination was not obtained, the calulated being 11.0 per cent. while 10.0 was found.

Acetylthioureaimidoisobutyl Benzoate, $C_6H_5C\begin{matrix}=NCSNHCOCH_3\\ \diagdown OC_4H_9\end{matrix}$.

—Benzimidoisobutyl ester and acetyl thiocyanate combine with evolution of considerable heat and, on cooling, the mixture completely solidifies, whereupon, on crystallizing from alcohol, colorless needles are obtained which melt from 125°–126°. A nitrogen determination gave:

	Calculated for $C_{14}H_{18}N_2O_2S$.	Found
Nitrogen	10.0	9.9

When this imido ester was treated in alcoholic solution with hydrogen chloride a compound was obtained melting at 166° and which had all the properties of *acetylthiourea*, $CH_3CONHCSNH_2$.

With phenylhydrazine it evolves hydrogen sulphide, and on crystallizing the product from alcohol acetylthiourea separates.

Benzoylthioureaimidoethyl Benzoate, $C_6H_5C\begin{matrix}=NCSNHCOC_6H_5\\ \diagdown OC_2H_5\end{matrix}$.

—When benzimidoethyl ester (6.1 grams) was mixed with 5.6 grams of benzoyl sulphocyanate the mixture completely solidified. On crystallizing from alcohol, needles were obtained which melted from 131°–132°, and on analysis the following result was obtained:

	Calculated for $C_{17}H_{16}N_2O_2S$.	Found.
Nitrogen	8.9	8.8

When this imido ester was warmed with phenylhydrazine the only crystalline material obtained was *benzoylthiourea*.

Benzoylthioureaimidoisobutyl Benzoate, $C_6H_5C(=NCSNHCOC_6H_5)(OC_4H_9)$.—When benzimidoisobutyl ester (10 grams) and benzoyl sulphocyanate (7.4 grams) were mixed, considerable heat was evolved and the mass solidified. When this was crystallized from alcohol, colorless needles, melting at 120,° were obtained. A nitrogen determination gave:

	Calculated for $C_{19}H_{20}N_2O_2S$.	Found.
Nitrogen	8.2	8.4

When this imido ester was treated with hydrogen chloride in benzene solution, *benzoylthiourea*, melting at 169°–170° was obtained. Apparently this same compound was obtained when the ester was treated with ammonia, phenylhydrazine and with benzoyl chloride. These results show the great tendency this ester has to separate at the double bond.

Benzoylthioureaimidoisobutyl Benzoate Methyl Iodide, $C_6H_5C(=NCSNHCOC_6H_5)(CH_2I)(OC_4H_9)$.—The above imido ester and methyl iodide combine in benzene solution to form a compound containing iodine and which crystallizes from alcohol in well-developed, colorless prisms melting from 204°–205.° A nitrogen determination gave:

	Calculated for $C_{20}H_{23}N_2O_2SI$.	Found.
Nitrogen	5.7	5.7

Benzenylbenzoylthioureaphenylamidine, $C_6H_5C(=NCSNHCOC_6H_5)(NHC_6H_5)$.—When molecular proportions of the above imido ester and aniline were heated in benzene solution a substance was obtained which crystallized in colorless prisms and which melted from 145°–146°. A nitrogen determination gave:

	Calculated for $C_{21}H_{17}N_3OS$.	Found.
Nitrogen	11.6	11.2

No hydrochloric acid salt was obtained when this amidine was treated with hydrogen chloride.

Benzenylbenzoylthioureaorthotolylamidine, $C_6H_5C\begin{matrix}=NCSNHCOC_6H_5\\ -NHC_6H_4CH_3\end{matrix}$.—This compound was obtained on fusing the imido ester with ortho toluidine. It crystallized from alcohol in colorless prisms which melted from 116°–117° and a nitrogen determination gave:

	Calculated for $C_{22}H_{19}N_3OS$.	Found.
Nitrogen	11.2	10.8

Benzoylthioureaimidomethylphenyl Acetate, $C_6H_5CH_2C\begin{matrix}=NCSNHCOC_6H_5\\ -OCH_3\end{matrix}$.—When phenylacetimidomethyl ester (5.6 grams) was treated with benzoylthiocyanate (5 grams), the mixture solidified immediately. It was crystallized from alcohol and obtained in the form of colorless needles melting from 116°–117°. A nitrogen determination gave:

	Calculated for $C_{17}H_{16}N_2O_2S$.	Found.
Nitrogen	8.9	9.3

When this imido ester was treated with phenylhydrazine, hydrogen sulphide was evolved but the product, on crystallizing from alcohol, was neither an amidine or a triazole. It contained 15.4 per cent. of nitrogen while the calculated for *benzoylthiourea* is 15.5 per cent. It agreed in all its properties with the acylthiourea.

Benzoylthioureaimidoethylphenyl Acetate, $C_6H_5CH_2C\begin{matrix}=NCSNHCOC_6H_5\\ -OC_2H_5\end{matrix}$, was prepared from phenylacetimidoethyl ester. It forms colorless needles from alcohol which melt from 140°–141°. When treated with hydrogen chloride in alcoholic solution it gave small prismatic crystals, melting from 169°–170°, and a nitrogen determination agreed with the calculated for benzoylthiourea:

	Calculated for $C_8H_8N_2SO$.	Found.
Nitrogen	15.5	15.6

The Thiocyanic Acid Salt of Benzimidoisobutyl Ester, $C_6H_5C(NH_2)(OC_4H_9)-SCN$, is readily formed on mixing aqueous solutions of the imido ester hydrochloride and potassium cyanate. It then separates as a mass of colorless needles or prisms which are quite difficultly soluble in water. It melts at about 130°. A portion dried in a desiccator over calcium chloride and then for a few minutes at 60°–70° gave the following results on analysis:

	Calculated for $C_{12}H_{16}N_2SO$.	Found.
Nitrogen	11.8	11.9

Its reaction with iron chloride and with alkali showed that this material was a salt of the imido ester and not the isomeric thioureaimidoisobutyl benzoate. It decomposes on warming with water smoothly into ammonium thiocyanate and isobutyl benzoate and when heated above its melting-point it gives benzamide and isobutyl thiocyanate.

EXPERIMENTS BY BAYARD BARNES.

Carbethoxythioncarbamic Ester, $C_2H_5OCONHCS.OC_2H_5$.—This was prepared according to the directions of Delitsch;[1] when purified by means of its potassium salt, it was found to boil unaltered at 135° at 13 mm. pressure. It melts at 44°.

1-Phenyl-3-ethoxy-5-triazolone, $C_6H_5N(OC-NH-C.OC_2H_5)=N$, was obtained when the above carbamic ester (15 grams) was heated with phenylhydrazine (9 grams) on the steam-bath. After the evolution of hydrogen sulphide and alcohol had ceased, the material was crystallized from hot water, whereupon colorless needles, melting from 150°–151°, were obtained. This material was soluble in alkali and was precipitated unaltered by acids. It contained no sulphur and on analysis the following results were obtained:

	Calculated for $C_{10}H_{11}N_3O_2$.	Found.
Carbon	58.5	58.9
Hydrogen	5.2	5.3
Nitrogen	20.5	20.4

[1] *J. prakt. Chem.*, 10, 118.

When this compound was boiled for some time with concentrated hydrochloric acid and alcohol, colorless plates were obtained, which melted at 263°, showing signs of melting at 255°. For purposes of comparison Pinner's[1] urazole was prepared and it was found to be identical with the above.

Acetylphenylurethane, $CH_3CO(C_6H_5)NCO.OC_2H_5$.—Ten grams of phenylurethane were boiled with an equal weight of acetic anhydride for several hours whereupon, on pouring into water, unaltered urethane was obtained. Acetyl chloride was then added and boiled for about one hour. Then about one-half of the solution was distilled off at ordinary pressure and the remainder at 10 mm. pressure whereupon the material practically all boiled from 142°–143°. It was thus obtained as a colorless oil which, like formylphenylurethane, refused to solidify on cooling. A nitrogen determination gave:

	Calculated for $C_{11}H_{13}NO_3$.	Found.
Nitrogen	6.7	7.3

When this compound was gently warmed with phenylhydrazine and the product was crystallized from benzene, colorless plates, melting at 128°, were obtained. The following nitrogen determination showed that this material was not a triazole derivative but rather *acetylphenylhydrazine*:

	Calculated for $C_8H_{10}N_2O$.	Found.
Nitrogen	18.6	18.0

The action of phenylhydrazine on Hugershoff's[2] *acetylphenylthiocarbamide*, $CH_3CONHCSNHC_6H_5$, melting at 139°, although some hydrogen sulphide was evolved, did not lead to the formation of a triazole derivative, at least not as the chief product. The material that separated, on crystallizing from alcohol, formed large, four-sided plates melting at 153° and having all the properties of *phenylthiourea*. In other words the action of phenylhydrazine, in this case, removes the acetyl group.

NEW HAVEN, CONN., May 29, 1900.

[1] *Loc. cit.*

[2] *Ber. d. chem. Ges.*, **32**, 3658.

THE NUCLEIC ACID OF THE EMBRYO OF WHEAT AND ITS PROTEIN COMPOUNDS.[1]

By Thomas B. Osborne and George F. Campbell.

Received June 16, 1900.

Frankfurt[2] has estimated the proportion of various constituents of the embryo of wheat and found globulin, 21.62 per cent., and albumose, 13.62 per cent.

O'Brien[3] states that the proteids of wheat germs consist of globulins of the myosin-type, coagulating at 55°, soluble in dilute solutions of sodium chloride or magnesium sulphate and precipitated by excess of these salts; globulins of the vitellin type, coagulating at 75°–78° and soluble in dilute solution of sodium chloride, but not precipitated by an excess; proteose; and albumin not coagulating below 80°, soluble in sodium chloride solution, not precipitated by an excess, nor by dialysis, nor by carbonic acid.

As neither of these investigators has given more than a superficial account of the protein[4] substances found by him in the embryo of wheat, we determined to investigate this subject, in order to learn definitely which of the proteins contained in the wheat kernel are predominant in the embryo, and whether there is any special difference in character between the proteins of the more physiologically active germ and those of the dormant endosperm.

As a result of this investigation we have found that, of the proteids described by one of us[5] as constituents of the wheat kernel, the albumin (leucosin), the globulin, and the proteose, which in the whole seed are present in small proportions, together constitute a large part of the embryo, and further, that gliadin and glutenin, the principal proteid constituents of the endosperm, are not present in the embryo.

[1] From advance sheets of the Report of the Connecticut Agricultural Experiment Station for 1899, communicated by the authors.

[2] *Versuchs-Stationen*, 47, 449.

[3] *Annals of Botany*, 9, 543.

[4] In this paper the term protein denotes the basic molecules which, combined with simple acids, form the "simple proteids," and combined with other more complex groups, form the "compound proteids" (Hammarsten, "Text-book of Physiology," translated by Mandel, third edition (1900.) The term proteid in this paper includes both of these groups of protein compounds.

[5] Osborne and Voorhees: *Am. Chem. J.*, 15, 392 (1893).

Although the globulin and leucosin obtained from the whole seed were free from phosphorus, all of our preparations of the globulin, and many of those of the albumin, from the embryo contained various yet considerable quantities of this element, and when digested with pepsin, yielded insoluble products having the characteristics of nuclein. The elementary composition of those preparations of the embryo-albumin which contained no phosphorus, or only traces, was the same as that of leucosin prepared from the whole seed, while the composition of those embryo-albumin preparations which contained phosphorus differed from that of leucosin in proportion to the phosphorus present.

Analyses of different preparations of embryo-globulin showed no agreement, even when corrected for ash.

These facts led us to examine our extracts for nucleic acid, and having found this acid and determined its composition, it was seen that the differences just alluded to disappear if we assume the phosphorus to be present as nucleic acid and calculate the analyses free from this acid and from ash.

In a paper recently published by one of us on "Some Definite Compounds of Protein Bodies"[1] it was shown that many, if not most preparations of so-called native proteids are, in fact, compounds of protein bodies with acids, and it was suggested that nucleoalbumins might prove to be similar phosphoric acid compounds.

In that paper it was also shown that the crystallized globulin, edestin, obtained from hemp-seed, forms a compound with one, and another compound with two molecules of hydrochloric acid, both of which are crystalline, and that the preparations of this globulin as commonly obtained are mixtures of these in various proportions. It was also shown that the protein molecule can combine with more than two molecules of acid, so that several multiple compounds of one acid with the same protein molecule can undoubtedly exist.

That our preparations from the wheat-embryo are mixtures of two or more compounds is shown by the variable proportions of phosphorus which they contain. That phosphorus is a chief factor in determining the proportion of nuclein that separates

[1] This Journal, 21, 486.

during pepsin digestion is shown by the fact that the amount of nuclein found is always in close relation to the amount of phosphorus contained in the preparation.

That the preparations are not mixtures of already formed nuclein with ordinary proteid matter is shown by the difference in solubility and behavior of the original preparation from that of the nuclein derived from it.

It would seem, then, that the nuclein obtained by pepsin digestion is not an original constituent of the extract nor of the cells of the embryo, but results through combination of several molecules of nucleic acid with one of protein.

Accordingly we conclude that these phosphorus-containing preparations from the wheat embryo are mixtures of different protein nucleates and that when subjected to pepsin digestion, in consequence of the conversion of a part of the protein substance into proteose, the proportion of nucleic acid to unaltered protein is increased, so that higher acid nucleates are formed which are insoluble in the digestive fluid.

The grounds for these conclusions are given in the following detailed account of our investigation.

The material at our command consisted of a quantity of wheat germs, a specially prepared product of the Pillsbury Mills, from which the bran and endosperm had been thoroughly removed, which was kindly procured for us by Mr. David Chidlow, of Chicago.

The germ meal, which was prepared and sent to us in cold weather, was immediately extracted with petroleum naphtha and ground to a flour.

I. The Nucleic Acid of the Wheat Embryo.

We shall later show that the precipitate produced by saturating the slightly acid aqueous extract of this wheat-embryo meal with sodium chloride contains almost all the phosphorus of the extract. We accordingly extracted a large quantity of the meal with water, saturated the extract with sodium chloride and subjected the precipitate to a vigorous pepsin digestion. We thus obtained a considerable quantity of nuclein from which we prepared nucleic acid in the following manner:

The nuclein was thoroughly washed with water and then

dilute potash solution was added until all the nuclein had dissolved and its solution become faintly alkaline to phenolphthalein.

This solution was cautiously treated with dilute hydrochloric acid until a precipitate was formed, which separated readily from the solution. This was filtered out and the clear filtrate found to yield no precipitate on adding a *little* more acid. A considerable quantity of strong hydrochloric acid was then added, causing a precipitate of nucleic acid, which separated in large flocks that rapidly settled to a coherent layer. The deposit continued to contract and soon became so dense and brittle that it could be ground to a powder even under water. The solution was decanted, the sediment was thoroughly washed, redissolved with alkali and again thrown down by adding acid. Since this last precipitate still contained much coloring-matter, it was again dissolved with alkali and the solution poured into alcohol. This retained the basic coloring-matter and threw down a voluminous precipitate of potassium nucleate, which was thoroughly washed with large quantities of alcohol, dried to remove the alcohol and then dissolved in water and the nucleic acid reprecipitated by an excess of hydrochloric acid. Since all the coloring-matter had not been removed by the preceding treatment, the precipitate was twice dissolved in alkali and precipitated by pouring into a large volume of alcohol. The nucleic acid was then thrown down by gradually adding dilute hydrochloric acid to the solution of the potash salt; after thoroughly washing the precipitated acid with water and with alcohol it was dried over sulphuric acid and found to weigh 10.14 grams.

After drying to constant weight in hydrogen at 100°, its weight remained unchanged on further heating at 110° in air. When thus dried it had the following composition:

	I.	II.
Carbon	36.18	36.31
Hydrogen	4.48	4.42
Nitrogen	16.03	16.10
Phosphorus	8.95	8.86
Ash	3.52	....
P_2O_5 in ash	2.88	....
Difference	0.64	....

The ash consisted, chiefly if not wholly, of potassium meta-

phosphate and therefore by subtracting from it the phosphorus pentoxide which it contained, we obtain the amount of inorganic base which had been precipitated as an acid salt together with the free nucleic acid. Calculating our analyses free from this base, we have the following figures:

COMPOSITION OF NUCLEIC ACID.

	Calculated for $C_{21}H_{31}N_8P_3O_{16}$.	Found.
Carbon	36.16	36.48
Hydrogen	4.45	4.48
Nitrogen	16.01	16.17
Phosphorus	8.89	8.96
Oxygen	34.49	33.91
	100.00	100.00

About 2 grams of this preparation were hydrolyzed by heating for an hour and a half in a boiling water-bath with 2 per cent. hydrochloric acid.

On adding ammonia to this solution, a precipitate soon separated which was digested on the water-bath with an excess of ammonia, filtered out, washed, dried over sulphuric acid, and found to weigh 0.27 gram.

This substance gave the murexide reaction and was insoluble in hot ammonia, both of which properties are characteristic of guanin. On analysis this crude guanin was found to contain:

	Calculated for guanin $C_5H_5N_5O$.	Found.
Carbon	39.74	40.96
Hydrogen	3.31	3.67
Nitrogen	46.36	45.21
Oxygen	10.59	10.16
	100.00	100.00

The solution filtered from the guanin was precipitated by cold ammoniacal silver nitrate and the voluminous, gelatinous precipitate washed, pressed on filter-paper, suspended in water, and decomposed by hydrochloric acid. The solution containing the hydrochloride of another base, was repeatedly evaporated with water to decompose the chloride and the free base found to weigh 0.2272 gram. This was again dissolved in water and the silver salt precipitated from a boiling solution, the silver compound

was decomposed with hydrochloric acid, and the solution of the hydrochloride was evaporated and crystallized.

The substance separated wholly in four-sided prisms, most of which were truncated by planes at right angles.

This hydrochloride was then converted into the picrate by dissolving in water, adding a little ammonia, evaporating to dryness, dissolving in about 100 cc. of water, and precipitating with a 1.1 per cent. solution of picric acid added cautiously. The very voluminous yellow precipitate was quickly filtered out with the help of a pump, washed thoroughly with water, and dried over sulphuric acid. We thus obtained 0.3766 gram of a picrate, which lost nothing on drying at 110° and had the following composition:

	Calculated for adenin picrate, $C_{11}H_8N_8O_7$.	Found.
Carbon	36.27	36.07
Hydrogen	2.19	2.51
Nitrogen	30.77	30.28
Oxygen	30.77	31.14
	100.00	100.00

From the behavior of this base and the composition of its picrate, it is evidently adenin. Since this acid yields on hydrolysis the purin bases, guanin and adenin, as well as phosphoric acid, there can be no doubt that it is a true nucleic acid closely related to the guanylic acid recently described by Bang.[1] The facts that we obtained these two bases from the nucleic acid in nearly molecular proportion and that almost all the nitrogen of the acid was recovered in the guanin and adenin separated from it, lead us to believe that both these bases exist together in the acid molecule. If such is the case, our formula already given must be multiplied by 2.5, making it $C_{89}H_{77}N_{29}P_5O_{37}$. This formula resembles that of guanylic acid, which, calculated to the same basis, is $C_{85}H_{75}N_{25}P_5O_{42}$. The two acids are different, since Bang's guanylic acid yields a pentose on hydrolysis, whereas we have obtained no evidence that any sugar can be derived from our acid. As we are at present engaged in a study of the reactions and constitution of this acid, we will reserve further statements respecting it for a future paper, which we expect to be able to publish soon.

[1] *Ztschr. phys. Chem.*, **26**, 133.

II. The Proteids of the Wheat Embryo.

THE AQUEOUS EXTRACT.

The germ flour, described on page 381, when treated with water, yields a gummy mass from which a clear extract is secured with difficulty. From 500 grams of meal an extract was obtained with 2,000 cc. of water, of which 1,400 cc. could be filtered clear. This extract was neutral to litmus, alkaline to lacmoid, and so acid to phenolphthalein that 19 cc. of decinormal alkali were required to neutralize 100 cc. of it to this indicator.

When a freshly prepared, dilute, aqueous extract of the recently ground wheat germs is heated in a water-bath, no coagulation occurs, the solution becoming slightly opalescent. If a more concentrated extract, such as may be obtained by treating 1 part of meal with 5 parts of water is thus heated, the entire solution solidifies to a firm, opaque jelly, free from visible particles. If to either of these solutions a very little hydrochloric acid is added before heating, an abundant flocculent coagulum separates on heating.

After standing awhile, the aqueous extract becomes gradually acid to limus, so that when heated slowly it becomes turbid at about 50° and a large flocculent coagulum separates at 55°. Heated to 65° for some time and filtered, a second coagulum may be obtained on raising the heat from 65° to 100°. The amount of this second coagulum is about one-third that of the first.

The coagulated proteid is dissolved by 0.5 per cent. potassium hydroxide solution, but not perceptibly by 0.4 per cent. hydrochloric acid solution, unless the latter is heated, when a clear transparent jelly is formed.

Freed from coagulable protein, the aqueous extract still contains a relatively large amount of substance which has the reactions of proteose.

When the concentrated aqueous extract is poured into a large volume of distilled water, a turbidity forms at first, which mostly disappears after shaking, indicating the absence of a notable quantity of globulin held in solution by the salts dissolved from the meal.

Saturation of the extracts with sodium chloride gives a considerable precipitate, only a small part of which can be redis-

solved in dilute salt solution. When this dissolved part is precipitated by again saturating with salt, it also is converted, to a large extent, into an insoluble form; the part still remaining in solution is, like a globulin, precipitated by dialysis.

When the solution saturated with sodium chloride is filtered, and the diluted filtrate saturated with ammonium sulphate, a part of the precipitate produced, when redissolved in water, is thrown out of solution by saturating with sodium chloride, though before precipitation with ammonium sulphate it dissolved in saturated sodium chloride solution.

These reactions show that changes occur which involve the albumin coagulating at 55°, for after freeing the extract from all protein precipitable by saturating with salt or by dialysis, there remains in solution only a small proportion of this albumin.

Thus, an aqueous extract corresponding to 666 grams of germ meal, when heated to 65°, yielded 62 grams of coagulum, or 9.3 per cent.; a similar extract on dialysis deposited 9.2 per cent., only 0.87 per cent. of coagulable and 2.0 per cent. of uncoagulable protein remaining in solution. The precipitate, produced by dialysis, was but slightly soluble in salt solution, having become largely coagulated. From these facts it is clear that one and the same protein substance gives rise to these apparently different protein bodies, and consequently the substance which O'Brien considered to be a globulin of the myosin type and an albumin, coagulating at 80°, are in fact derivatives of the same protein substance.

The cause of these changes was not determined, though it seems most probable that they are the result of a slow development of acid in the extract, which, by uniting to the protein in increasing proportions, forms chemically different substances. Such a development of acid takes place rapidly in muscle plasma, under the influence of which quite similar changes in the proteins there present can be observed.

Why Frankfurt overlooked albumin, present in such large proportion in the aqueous extract, is not easily understood, unless, before heating his solutions, he either added no acid or too much, so that he converted this substance into an uncoagulable acid compound.

Hydrochloric acid added to the extract in very small quantity

causes a flocculent coagulum to separate on heating, while a slightly larger quantity, added before heating, entirely prevents the formation of this coagulum. Acetic acid and nitric acid give precipitates in the extracts which are not soluble in a reasonable excess of either of these acids.

In order to determine definitely the relations of these variously obtained substances, we have made a large number of fractional precipitations under quite different conditions, an account of which we now give:

An extract was made by treating 700 grams of germ meal with seven times its weight of water, straining through bolting-cloth and filtering the liquid perfectly clear. This was slightly colored, perfectly neutral to litmus, alkaline to lacmoid, and strongly acid to phenolphthalein. A portion of it was at once heated for one hour in a water-bath at 60°, and the large coagulum produced was filtered out, washed thoroughly with hot water and with alcohol and dried over sulphuric acid, giving 24 grams of preparation 1.

Another preparation was made by heating in a water-bath at 65°, 2,000 cc. of a clear aqueous extract, obtained by treating 3,000 grams of the germ meal with 9,000 cc. of water. The coagulum produced, when washed with hot water and alcohol and dried over sulphuric acid, weighed 62 grams, forming more than 9 per cent. of the oil-free germ meal. This is preparation 2.

Another aqueous extract was heated at 65°, until all the proteid coagulable at this temperature had separated. The coagulum produced, when washed with hot water and alcohol was dried over sulphuric acid and found to weigh 16.68 grams. The filtrate from this coagulum, heated in a boiling water-bath, yielded a second coagulum which, when washed and dried, formed preparation 3, weighing 4.9 grams.

A portion of the extract, which yielded preparation 1, was saturated with ammonium sulphate; the resulting precipitate was dissolved as far as possible in water, its solution filtered clear, and dialyzed for four days. During this time a considerable precipitate formed, that, when filtered out, was found to be insoluble in salt solution. The solution, filtered from that substance and dialyzed in running water until nothing more sepa-

rated, was filtered and heated at 60°, which caused a coagulum. This coagulum, washed with hot water and with alcohol and dried over sulphuric acid, weighed 7.1 grams and made preparation **4.**

In a clear water extract of wheat germ meal, dialyzed four days, there appeared a dense turbidity, due apparently to a globulin, since it dissolved on adding sodium chloride. Passing carbon dioxide gas through the dialyzing solution seemed to increase the turbidity, but effected no definite separation. As it was found that 10 cc. of N/10 hydrochloric acid per 100 cc. of the extract caused a separable precipitate, this proportion was added and the resulting flocculent precipitate brought into solution again by adding salt. The clear extract was then dialyzed for two days in running water and filtered from an amorphous precipitate, which was treated as later described on p. 400.

The filtrate from this precipitate was further dialyzed for three days more in running water and then, as nothing separated, for four days more into alcohol. The precipitate which resulted was dried over sulphuric acid, exhausted with water, in order to remove all uncoagulated proteids, as well as other soluble substances, dehydrated with absolute alcohol, again dried and weighed, yielding 12 grams of preparation **5.**

Another aqueous extract was saturated with pure sodium chloride, the abundant precipitate filtered out, treated with dilute brine and the resulting solution filtered from a relatively considerable quantity of insoluble matter. This filtrate was saturated with sodium chloride, a second precipitate filtered out and likewise treated with dilute salt solution. The insoluble portion was removed by filtration and the clear filtrate dialyzed. The small precipitate separated by dialysis was washed and dried, weighed 4.8 grams, and formed preparation **6.**

The filtrate from the first precipitation of the substance of preparation **6,** caused by saturating its solution with sodium chloride, as described above, was diluted with water and saturated with ammonium sulphate. The precipitate which resulted was dissolved in water and its solution precipitated by saturating with sodium chloride. Although this substance had previously been soluble in saturated brine, after precipitation with ammonium sulphate it was found to be nearly all insoluble

therein, so that almost complete precipitation resulted on again saturating with sodium chloride. The precipitate so produced was filtered out, dissolved in dilute salt solution, and reprecipitated by dialysis. We thus secured 7.6 grams of preparation 7.

By saturating another aqueous extract of germ meal with sodium chloride a very large quantity of proteid was separated which was filtered out, exhausted with dilute salt solution and washed thoroughly with water and alcohol. Dried over sulphuric acid, the preparation, 8, weighed 17 grams.

The filtrate and saline washings from preparation 8 were united and again saturated with salt, and yielded a small precipitate which, dissolved in brine and precipitated by dialysis, gave preparation 9, weighing 2.8 grams. As the salt-saturated solution from which this preparation had separated contained so little protein, it appears that nearly all the proteid precipitated from the aqueous extract by saturating with salt had been converted into the insoluble substance forming preparation 8.

The filtrate, from the salt-saturation precipitate produced in the aqueous extract, was dialyzed in water for several days and the still clear solution then dialyzed in alcohol for twenty-four hours. The proteid, thereby precipitated in a coagulated state, was filtered out, washed with water and then with alcohol, yielding 12.4 grams of preparation 10.

Another aqueous extract was saturated with sodium chloride and the precipitate, treated in the same way as preparation 8, yielded 18 grams of preparation 11.

The saline washings of the last preparation were dialyzed free from chlorides and gave a precipitate weighing 2.86 grams when washed and dried, which formed preparation 12, having the properties of a globulin, dissolving readily on adding salt and being precipitated from such solution by water.

The filtrate from the final precipitation of 12, when heated in a boiling water-bath, gave a coagulum which formed preparation 13, weighing 1.64 grams.

The salt-saturated filtrate from the first precipitation of 11, as already described, was heated to boiling and the coagulum produced was filtered out, giving preparation 14, weighing 5.47 grams.

Since analysis showed that most of the preparations already

described contained phosphorus, some even in large amount, we made an attempt to separate the phosphorus from our extract, in order to determine, if possible, the relation of the preparations free from phosphorus to those which contained much phosphorus.

Two thousand grams of meal were treated with 6 liters of distilled water and the extract (4 liters) was squeezed out as completely as possible in a press.

As a preliminary experiment, 100 cc. of this clear, filtered extract were made faintly alkaline to phenolphthalein, with about 40 cc. of N/10 potassium hydroxide solution. To insure a sufficient quantity, 20 cc. more of alkali were added and thereupon a little calcium chloride, which gave a precipitate that seemed to partly dissolve on adding sodium chloride. The undissolved part, when washed with dilute salt solution, water, and alcohol and dried, weighed 1.7 per cent. of the meal, contained about 55 per cent. of organic matter, and left 45 per cent. of ash, consisting of tricalcium phosphate.

To 2,000 cc. of the original extract were then added 1,350 cc. of a solution containing alkali equivalent to 1,560 cc. N/10 solution, with sodium chloride enough to form 6.5 per cent. of the total liquid. To this, a solution of calcium chloride was added, as long as a precipitate formed, and after standing over night the solution was decanted from the precipitate and filtered clear on a pulp filter. Of the clear filtrate, 2,200 cc. were made as neutral as possible to *litmus*, by adding 180 cc. of N/10 hydrochloric acid solution. Of the thus neutralized solution, 1,000 cc., when gradually heated in a water-bath, became turbid at 52° and a considerable coagulum separated at 53°. After the temperature had been slowly raised to 65° and kept at this point for some time, the coagulum was filtered out, washed and dried as usual, giving preparation **15**, weighing 6.4 grams. Another portion of this extract, filtered from the calcium chloride precipitate, was saturated with ammonium sulphate while still slightly alkaline to litmus, the resulting precipitate filtered out, dissolved in water, its solution filtered clear and dialyzed. A slight precipitate formed on dialysis, which was removed by filtering, the solution was heated in a boiling water-bath and the proteid thus coagulated was filtered out, washed, dried, and weighed as usual, giving 3.07 grams of preparation **17**.

To determine what effect the removal of the phosphorized substance thrown out by calcium chloride had upon precipitation with salt, we made neutral to litmus a liter of the filtrate from the calcium chloride precipitate and then saturated with sodium chloride. The large precipitate which formed was washed by decantation with water, in which it gradually dissolved, until only an insignificant quantity remained. The similarly obtained precipitate from the simple aqueous extract we have shown on page 389, to be nearly all insoluble in water.

To separate globulin from the aqueous extract, 1,200 cc. of clear, filtered extract were obtained from 200 grams of the germ meal treated with 2,000 cc. of water. One liter of this extract was dialyzed in running water for six days, and the large precipitate resulting filtered out, washed with water and alcohol, and dried over sulphuric acid, giving preparation 18, weighing 9.17 grams.

These preparations, thus variously obtained from the aqueous extract, were dried to constant weight at 110° and analyzed with the following results, most of the figures given in the table being the average of closely agreeing duplicate determinations:

TABLE I.—COMPOSITION OF PREPARATIONS OF PROTEID FROM THE WATER EXTRACT OF THE WHEAT EMBRYO.

	1	2	3	4	5	6	7	8	9
Carbon.....	51.13	50.52	50.17	52.39	51.77	52.13	52.73	43.59	52.28
Hydrogen..	6.85	6.81	7.01	6.83	6.81	7.04	7.11	5.77	6.97
Nitrogen...	16.28	16.47	16.66	16.20	16.11	16.48	16.00	15.16	16.38
Sulphur....	1.18	1.17	1.00	1.32	1.30	1.49	1.53	0.90	1.39
Phosphorus	0.72	0.97	0.91	trace	0.17	0.06	none	3.38	0.07
Ash.....	2.73	2.90	3.03	0.35	1.39	0.43	0.39	13.04	0.44
P_2O_5 in ash	1.88	2.09	1.91	trace	0.47	trace	none	6.73	trace
	10	11	12	13	14	15	16	17	18
Carbon.....	51.21	46.67	51.87	....	51.95	51.65	....	52.02	49.59
Hydrogen..	6.85	6,19	6.89	....	6.86	6.66	....	7.00	6.68
Nitrogen...	16.18	15.89	16.65	16.31	16.08	16.02	16.09	16.45	16.34
Sulphur....	1.10	0.93	1.19	1.35	1.60	1.13	1.12	1.24	0.91
Phosphorus	0.46	2.53	trace	trace	trace	trace	trace	none	1.85
Ash.....	2.19	8.17	0.38	0.45	0.32	1.09	2.83	0.56	2.50
P_2O_5 in ash	1.11	5.71	trace	trace	trace	trace	trace	none	1.79

Assuming that those of the foregoing preparations which contain phosphorus are compounds of protein with the nucleic acid,

which was separated from the aqueous extract of wheat germs and the composition of which is given on page 383 of this paper, and also assuming that all the phosphorus of these preparations is a part of the nucleic acid, we have calculated the composition of these preparations free from nucleic acid. The analyses were further calculated ash-free by subtracting the phosphorus pentoxide contained in the ash from the total ash, which seems permissible since the ash consisted almost wholly of metaphosphates of potassium and sodium, strongly indicating that the phosphorus pentoxide was derived from the nucleic acid. These calculations gave the following results:

TABLE II.—COMPOSITION OF LEUCOSIN CONTAINED IN THE PREPARATIONS FROM WATER EXTRACTS OF THE WHEAT EMBRYO.

	1	2	3	4	5	6	7	8	9
Carbon	52.93	52.75	52.41	52.57	52.57	52.47	52.93	53.23	52.64
Hydrogen..	7.12	7.16	7.38	6.85	6.91	7.08	7.13	7.09	7.02
Nitrogen...	16.45	16.68	16.94	16.26	16.27	16.55	16.06	16.30	16.46
Sulphur ...	1.29	1.32	1.13	1.32	1.34	1.50	1.53	1.60	1.41
Oxygen....	22.21	22.09	22.14	23.00	22.91	22.40	22.35	21.78	22.47
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

	10	11	12	13	14	15	16	17	18
Carbon	52.63	52.44	52.06	....	52.11	52.16	....	52.30	53.45
Hydrogen..	7.06	7.10	6.92	....	6.88	6.73	....	7.04	7.30
Nitrogen...	16.40	16.26	16.71	16.38	16.13	16.20	16.56	16.54	16.57
Sulphur....	1.17	1.34	1.19	1.35	1.60	1.14	1.15	1.24	1.16
Oxygen	22.74	22.86	23.12	....	23.28	23.77	....	22.88	21.52
	100.00	100.00	100.00		100.00	100.00		100.00	100.00

Of these, preparation **1**, **2**, **3**, **4**, **13**, **14**, **15**, **16**, and **17** were obtained by coagulation with heat, **5** and **10** by coagulation with alcohol, **8** and **11** by saturation with sodium chloride, **6**, **7**, **9**, and **12** by dialyzing salt solutions in water, and **18** by direct dialysis of the aqueous extract. Since some of these preparations formed the whole of the precipitable proteid contained in the extract, while others represented fractions, it is evident that all contain one and the same protein substance mostly combined with various proportions of nucleic acid.

Eliminating the nucleic acid, it thus appears that the composition of the protein part of those preparations which contain phosphorus is the same as that of the phosphorus-free proteid

preparations, although the former contain from very little up to more than 37 per cent. of nucleic acid.

Most of these preparations might, in accordance with custom, be called nucleoproteids, while 8 and 11 are, both in properties and composition, very much like nuclein. It is thus evident that these nucleoproteids and nucleins are nucleic acid compounds of protein which, owing to the high molecular weight of the nucleic acid, are more readily recognized as compounds than are those with acids of low molecular weight.

It is to be noted that these preparations show very diverse properties: some being like albumin; some like globulin; some being precipitated by saturation with salt, while others are not. As we have shown, these different properties are the result of changes caused by varying the conditions under which the proteid exists in the extract, and depend chiefly on the degree of acidity of the extract, whereby the numbers and kinds of acid molecules that combine with the protein molecule are altered.

Whatever may be the true cause of these changes, it is evident from the results here described, that the distinctions heretofore made between globulin and albumin, myosin and vitellin, etc., have very little value as a basis for classifying protein substances. This explains the difference between O'Brien's classification of leucosin as a myosin-like globulin, to which reference was made at the beginning of this paper, and our designation of it as an albumin, because of the ready solubility in water and coagulability by heat of the preparations which we had made.

Thus, preparation 18, weighing 9.17 grams, was insoluble in water and in salt solution and was not a precipitate of globulin, since in the filtrate from which it had separated on dialysis, only 0.87 gram of coagulable albumin was found instead of 9.5 grams as usually found by direct coagulation of the aqueous extracts; moreover the analysis shows it to be a compound of leucosin with 20 per cent. of nucleic acid.

On the preceding pages, it was shown that a small part of the precipitate, produced by saturating the aqueous extract with sodium chloride, is soluble in dilute salt solution and can be precipitated from this solution by dialysis, as a globulin-like substance, readily soluble again in salt solution. The precipitates thus obtained contain little or no nucleic acid, and have

very nearly the same elementary composition as leucosin, of which they are evidently compounds with a small proportion of some body of low molecular weight.

It is plain from these facts that O'Brien's myosin contains the same protein substance as my leucosin.

O'Brien's "*albumin*" coagulating at 75°–80° is unquestionably more of this same leucosin as shown by preparation 3, which formed about 25 per cent. of the total coagulable proteid. It has been the writer's experience that complete coagulation, especially in a solution quite free from salts, can be effected, if at all, only by heating the solution much above the lower coagulation temperature of the proteid to be separated.

From the whole seed we obtained leucosin with the same composition and general properties as from the embryo, but our preparations from the whole seed were free from phosphorus. This was probably because the proportion of nucleic acid to protein matter was smaller in the whole seed than in the embryo so that on extracting with water the nucleic acid did not form soluble compounds with the leucosin, but remained undissolved in combination with protein. In the following table is given the average of analyses of leucosin from the cereals.

TABLE III.—COMPOSITION OF LEUCOSIN PREPARED FROM VARIOUS CEREALS.

	Wheat embryo.	Wheat kernel.	Rye kernel.	Barley kernel.	Barley malt.	Maize[1] kernel.
Carbon	52.65	53.02	52.97	52.81	53.07	52.72
Hydrogen....	7.04	6.84	6.79	6.78	6.72	7.05
Nitrogen	16.43	16.80	16.66	16.62	16.71	16.82
Sulphur	1.32	1.28	1.35	1.47	} 23.50	1.32
Oxygen.....	22.56	22.06	22.23	22.32		22.05
	100.00	100.00	100.00	100.00	100.00	100.00

In an earlier paper on the "Chemical Nature of Diastase"[2] we pointed out that diastatic action appeared to be always associated with leucosin. Since our extracts of wheat embryo were so rich in leucosin, we determined the diastatic power of the

[1] This proteid was described by Chittenden and Osborne (*Am. Chem. J.*, **13**, 327) as a myosin-like globulin, and was later (This Journal, **19**, 525) designated maysin by the writer. Since we now find that leucosin may form compounds having the properties of globulin, it is probable that maysin and leucosin contain one and the same protein substance.

[2] This Journal, **18**, 542; Report Conn. Agr. Exp. Sta., 1895, p. 239.

germ meal by extracting with four times its weight of water and found that, under the conditions of Lintner's test, 0.10 cc. of the extract so made, when added to 10 cc. of a 2 per cent. solution of soluble starch, formed within one hour, at 20°, enough sugar to reduce 5 cc. of Fehling's solution. The 0.10 cc. of extract corresponds to 25 milligrams of the germs, from which it is seen that this meal possesses high diastatic power, though it is inferior in this respect to active malt.

SODIUM CHLORIDE EXTRACT.

Wheat germ meal treated with 10 per cent. sodium chloride brine forms a dense jelly-like mass from which it is nearly impossible to separate the solution.

With 3 per cent. brine a manageable extract can be made by using from six to ten times as much solvent as meal. Thus, 100 grams of the meal treated with 600 cc. of 3 per cent. salt solution yielded in fifteen hours 400 cc. of clear filtrate. As has just been shown, the aqueous extract on dialysis, in consequence of a change which affects leucosin, deposits a large amount of proteid, chiefly in the coagulated form. In order to obtain preparations of the proteid substance soluble in salt solutions, but insoluble in water, which should be free from this coagulable albumin, we treated 2,000 grams of germ meal with 20 liters of 3 per cent. salt solution heated to 70°, whereby the leucosin was coagulated and the salt-soluble globulin brought into solution. The extract, neutral to litmus, was filtered clear, at once saturated with ammonium sulphate and the proteids thus precipitated collected on a filter, dissolved in water, and the clear solution dialyzed in running water.

Proteid matter separated on dialysis in spheroids which, like legumin, conglutin, and amandin, united to a plastic mass on the bottom of the dialyzer.

This precipitate was dissolved in brine, filtered absolutely clear, dialyzed for 48 hours, the large precipitate which separated allowed to settle, and the solution, which was nearly free from protein, decanted.

A portion of the precipitate was washed first with water, which rendered it opaque and dense, then with dilute and finally with absolute alcohol and dried over sulphuric acid. This weighed

5.22 grams, and is preparation **19.** The rest of the precipitate was completely dissolved in 125 cc. of 10 per cent. salt solution. To this, water was added until its volume was 425 cc., thus making a salt solution of nearly 3 per cent. From this diluted solution a gummy deposit separated from which the liquid was soon completely decanted. The latter was further diluted with 325 cc. of water and the precipitate which resulted allowed to settle to a viscid transparent deposit. From this precipitate the solution was again decanted and dialyzed for 48 hours, but not more than a trace of globulin was deposited. The two precipitates produced by dilution were thoroughly washed with water and alcohol, dried over sulphuric acid and formed preparations **20** and **21,** weighing respectively 11.4 grams and 8.15 grams. A part of each of these preparations was set aside for analysis and the rest, dissolved together in 10 per cent. salt solution, allowed to stand over night at 4°. The solution was then decanted from a slight sediment, filtered clear and heated to 80° in order to coagulate any leucosin which might be present, and after two hours filtered from a very small coagulum which had gradually formed.

This filtrate was dialyzed in water for four days and the globulin which separated was washed with water and with alcohol and dried over sulphuric acid, giving preparation **22.**

The solution filtered from the first dialysis precipitates which yielded preparations **19, 20,** and **21;** was further dialyzed; a little globulin, which separated, was filtered out and the filtrate dialyzed into alcohol for four days. A precipitate was produced which, when washed with absolute alcohol and dried, weighed 25 grams. This substance consisted of proteid which will be described later, on page 402. Another series of fractional precipitations of this globulin-like proteid was made by extracting four kilograms of the oil-free germ meal with 27 liters 3 per cent. brine, heated to 67° at the time it was applied to the meal. The mixture was thoroughly stirred and thrown on filters. A clear filtrate of about 12 liters was finally obtained, which was saturated with ammonium sulphate. The precipitate produced was dissolved in water and its solution dialyzed for forty-eight hours, whereupon a large quantity of spheroids separated which on settling united to a coherent mass. This pre-

cipitate was washed by decantation with water, dissolved in brine, and its solution made faintly alkaline to litmus by cautiously adding N/10 potassium hydroxide solution. In order to separate phosphoric acid, a little calcium chloride solution was then added to this very slightly alkaline liquid and the latter, though apparently free from any precipitate of calcium phosphate, was filtered, whereby a little suspended matter was removed. The solution was made exactly neutral to litmus by adding 56 cc. N/10 hydrochloric acid and dialyzed for eighteen hours. A gummy precipitate, A, adhering to the bottom of the dialyzer, then separated, from which the solution, B, was decanted almost completely.

The precipitate, A, was dissolved in about 200 cc. of 5 per cent. brine and the liquid was poured into 800 cc. of water. The resulting flocculent precipitate settled rapidly to a coherent deposit from which the solution was decanted. The deposit was repeatedly washed by decantation with water, which caused it to lose its gummy character and become opaque, white, and granular. After dehydrating with absolute alcohol and drying over sulphuric acid it weighed 15.5 grams and was marked preparation **23**. The solution marked B was further dialyzed for forty-eight hours when a second precipitate formed, which, like **23**, completely dissolved in brine, to a solution perfectly neutral to litmus. This precipitate was washed by decantation with water, but the finer part settled so slowly that it was necessary to decant it together with the water. The sediment after exhausting with absolute alcohol and drying, weighed 23.5 grams, and formed preparation **24**. On long standing, the decanted washings deposited the finely divided matter, which was then collected on a filter, dissolved in brine and its solution precipitated by water, giving 15.4 grams of preparation **25**.

To determine the quantity of globulin contained in our oil-free germ meal, we treated 200 grams of the meal with 2,000 cc. of 3 per cent. salt solution heated to 65° and filtered the extract perfectly clear. Of this, 1,000 cc. were dialyzed until free from chlorides, when the precipitate of spheroids was filtered out, washed with water and with alcohol and dried over sulphuric acid. This preparation, **26**, formed 5.05 per cent. of the oil-free meal.

To obtain a quantity of this globulin for digestion with pepsin, a quantity of germ meal was extracted with 3 per cent. salt solution heated to 70°, the extract was filtered clear and saturated with ammonium sulphate. The precipitate produced was dissolved in water and the resulting gummy and somewhat turbid solution filtered clear. The filtrate was dialyzed until the solution gave no turbidity on pouring into distilled water. The proteid, which had then separated in spheroids, was filtered out, washed by decantation with water and with alcohol and dried over sulphuric acid, giving 27.3 grams of preparation **27**.

A part of the extract from which **27** had been prepared was mixed with an equal volume of N/10 potassium hydroxide, about twice the quantity necessary to neutralize the extract to phenolphthalein. The solution was then dialyzed in distilled water frequently renewed and in this way a considerable quantity of phosphorus was separated in the alkaline dialyzate. When all, or nearly all, which it was possible to separate in this way, had been removed, the solution in the dialyzer was neutralized with N/10 hydrochloric acid until it no longer reacted alkaline to litmus. This caused a turbidity. The acid was then further added until an acid reaction with litmus was obtained, producing a precipitate from which, after settling, the solution was decanted. The precipitate was then dissolved in brine, its solution filtered clear and dialyzed, whereby a substance was precipitated in spheroids, which was filtered out, washed with water and alcohol and formed preparation **28**, weighing 3 grams.

These preparations had the following composition:

TABLE IV.—COMPOSITION OF PREPARATIONS EXTRACTED BY SODIUM CHLORIDE SOLUTIONS FROM THE WHEAT EMBRYO.

	19	20	21	22	23	24	25	26	27	28
Carbon	...	...	48.77	50.03	50.23	48.17	49.39	48.75	49.79	48.67
Hydrogen	...	...	6.44	7.04	6.89	6.54	6.78	6.52	6.76	6.56
Nitrogen	18.14	18.21	18.12	18.39	18.23	18.06	17.95	18.16	18.01	17.97
Sulphur	0.49	0.56	0.51	0.60	0.53	0.55	0.48	0.63	0.61	0.61
Phosphorus	1.15	1.03	1.35	0.76	0.56	1.41	1.17	1.41	1.11	1.55
Ash	2.29	1.86	2.25	1.30	1.22	3.85	2.60	2.66	1.11	2.94
P_2O_5 in ash	1.66	1.34	1.68	0.84	0.80	2.00	1.82	2.00	0.68	2.30

These analyses, when calculated free from nucleic acid and ash, as was done for the albumin preparations, in the manner described on page 392, gave the following results:

TABLE V.—COMPOSITION OF THE GLOBULIN CONTAINED IN THE PREPARATIONS EXTRACTED FROM THE WHEAT EMBYRO BY SODIUM CHLORIDE SOLUTION.

	19	20	21	22	23
Carbon	....	....	51.37	51.58	51.40
Hydrogen	....	....	6.83	7.31	7.08
Nitrogen	18.59	18.59	18.62	18.70	18.45
Sulphur	0.57	0.63	0.60	0.66	0.57
Oxygen	....	....	22.58	21.75	22.50
			100.00	100.00	100.00
	24	25	26	27	28
Carbon	51.56	51.86	51.40	51.98	51.70
Hydrogen	7.07	7.19	6.94	7.12	7.05
Nitrogen	18.85	18.41	18.71	18.37	18.53
Sulphur	0.67	0.55	0.75	0.70	0.75
Oxygen	21.85	21.99	22.20	21.83	21.97
	100.00	100.00	100.00	100.00	100.00

These figures plainly show that our globulin preparations are mixtures of nucleates of one and the same protein substance and contain from 5 to 15 per cent. of nucleic acid. The preparations contain the same protein as the globulin which one of us has previously described as occurring in the kernel of wheat, rye, barley, and maize. In the entire kernel, so little of this globulin is present that it is difficult to prepare it pure therefrom. For this reason, we think, the analyses given below do not agree as closely as they might otherwise be expected to. From the whole seed this globulin is obtained entirely free from phosphorus, which we attribute to the much greater proportion of proteid matter to nucleic acid, in the seed, compared with that existing in the wheat embryo.

TABLE VI.—COMPOSITION OF THE GLOBULIN CONTAINED IN VARIOUS CEREALS.

	Wheat embryo.	Wheat[1] kernel.	Rye[2] kernel.	Maize[3] kernel.	Barley[4] kernel.
Carbon	51.57	51.03	51.19	51.99	50.88
Hydrogen	7.07	6.85	6.74	6.81	6.65
Nitrogen	18.60	18.39	18.19	18.02	18.10
Sulphur	0.65	0.65	23.88	0.66	24.37
Oxygen	22.11	23.08		22.52	
	100.00	100.00	100.00	100.00	100.00

[1] *Am. Chem. J.*, 15, 392.

[2] This Journal, 17, 429; also Report of Conn. Expt. Station for 1894, p. 147.

[3] *Am. Chem. J.*, 13, 327, 385 and 15, 20.

[4] This Journal, 17, 539; also Report of Conn. Expt. Station for 1894, p. 165.

Having determined the composition of this globulin-like proteid and also that of the albumin, it became clear that several preparations obtained from the aqueous extract were mixtures of these two substances, thus showing the globulin to be present to some extent in the aqueous extract.

As noted on page 388, when 2,000 cc. of an aqueous extract of about 650 grams of the meal were dialyzed in running water for four days, a dense turbidity was formed which could not be removed by filtration. This, however, on adding a little hydrochloric acid, was converted into a precipitate, which was readily dissolved by adding sodium chloride sufficient to make a 3 per cent. solution, and was precipitated from this solution by dialysis. We thus obtained 9 grams of preparation **29**, which, dried at 110°, had the following composition:

COMPOSITION OF PREPARATION 29.

	I.	II.	Av.	Corrected for ash and nucleic acid.	Calculated for 60 per cent. globulin, 40 per cent. leucosin.
Carbon	48.30	47.92	48.11	51.70	51.95
Hydrogen	6.49	6.41	6.45	7.07	7.07
Nitrogen	17.40	17.24	17.32	17.74	17.74
Sulphur	0.83	0.85	0.84	1.08	0.91
Phosphorus	1.91	....	1.91	....	....
Oxygen	....	....	....	22.51	22.53
Ash	3.95				
P_2O_5 in ash	2.95			100.00	100.00

This analysis corresponds pretty nearly with that of a mixture of 60 per cent. of the globulin with 40 per cent. of leucosin, except that the amount of sulphur found was somewhat greater than that calculated.

After heating another portion of the same aqueous extract to 65° for some time and filtering off the coagulum, the filtrate was dialyzed for five days into alcohol and the precipitate thereby produced filtered out and exhausted with water. The residue of proteid matter coagulated by alcohol, weighing 6.7 grams and marked preparation **30**, was then dried at 110° and analyzed with the following results:

COMPOSITION OF PREPARATION 30.

		Corrected for ash and nucleic acid.	Calculated for globulin 40 per cent., leucosin 60 per cent.
Carbon	49.49	51.80	52.13
Hydrogen	6.81	7.14	7.03
Nitrogen	16.87	17.32	17.30
Sulphur	0.93	1.14	1.05
Phosphorus	0.89	....	....
Oxygen	....	22.60	22.49
Ash	4.00	———	———
P_2O_5 in ash	2.01	100.00	100.00

This analysis corresponds quite nearly with that of a mixture of 40 per cent. of the globulin with 60 per cent. of the albumin.

THE PROTEOSE OF THE WHEAT EMBRYO.

In making the preparations already described considerable quantities of crude proteose were obtained from both the aqueous and sodium chloride extracts. After the leucosin and the globulin had been separated as completely as possible, the solutions containing the proteoses, as well as the unseparated residues of other proteids, were dialyzed into alcohol and the precipitates produced washed and dried over sulphuric acid.

A mixture, weighing 15.4 grams, was made by uniting several such preparations that had been obtained from aqueous extracts from which most of the other proteids had been separated, without heat, by saturating with sodium chloride and dialysis into alcohol. The mixture contained much matter made insoluble in water by the final treatment with alcohol. This was filtered out, washed thoroughly with water and with alcohol and when dried weighed 4.18 grams, and was marked preparation 31. The filtrate from this was saturated with ammonium sulphate, the precipitate redissolved and again precipitated in the same way. The solution of the second precipitate was dialyzed in cold distilled water until free from sulphate, and then for several days in alcohol. The precipitate thus produced was dissolved in water, a little insoluble matter filtered out, and its clear solution saturated with sodium chloride, which produced a small precipitate. This was filtered out, dissolved, and its solution dialyzed in water. The salt-saturated filtrate was likewise dialyzed and when both solutions were free from chlorine the dialyzers were

transferred to alcohol and the proteose thereby precipitated. The proteose separating on saturation with salt, gave 0.6 gram of preparation 32; that from the salt-saturated solution, 0.97 gram of preparation 33. This small yield of proteose indicates great impurity of the original crude product and shows that the proportion of proteose to other proteids is very small.

Another crude product was obtained by dialyzing into alcohol an aqueous extract, after separating leucosin which had been coagulated by heat. This, weighing 35 grams, was dissolved in water and the insoluble matter filtered out, washed and dried, giving preparation 34, weighing 7.26 grams.

The filtered solution was saturated with ammonium sulphate, the precipitate dissolved in water, and the clear solution dialyzed in distilled water until free from sulphates, and then in alcohol. The substance thus separated was again dissolved in water, and its solution saturated with salt; the precipitate thus produced was dissolved in water and its solution, as well as the salt-saturated filtrate, were dialyzed in water. When free from chlorine, these solutions were dialyzed in alcohol and yielded, respectively, preparations 35, weighing 4 grams and 36, weighing 1.84 grams.

Another preparation of crude proteose was obtained by extracting the meal with 3 per cent. sodium chloride solution heated to 70°, dialyzing the extract in water, coagulating the leucosin by heat and precipitating the proteose by dialysis in alcohol. A mixture of such preparations, weighing 31.6 grams, was treated with water, the insoluble matter filtered out, washed and dried, giving 5.16 grams of preparation 37.

The filtered solution was saturated with ammonium sulphate, the precipitate dissolved in water, the solution dialyzed in distilled water till free from sulphate, and then in alcohol. The separated proteose was redissolved in water and its solution saturated with sodium chloride. The precipitate which resulted was filtered out, dissolved in water and its solution, as well as the salt-saturated filtrate, was dialyzed in water till free from chlorine, and finally in alcohol.

The products thus obtained, formed, respectively, preparations 38, weighing 0.75 gram and 39, weighing 1.35 grams. One other proteose preparation was made from the aqueous

extract previously described on page 390 from which the phosphorus was largely separated by making it slightly alkaline and adding calcium chloride. After heating the extract to boiling and filtering out the coagulum, the filtrate was dialyzed into alcohol, the resulting precipitate dehydrated with absolute alcohol, dried over sulphuric acid, redissolved in water, and precipitated by saturating with ammonium sulphate. The gummy precipitate, having the general appearance and properties of similar precipitates of the proteoses obtained by the action of pepsin, was dissolved in water, dialyzed free from sulphates, and then precipitated by dialysis in alcohol, giving 2 grams of preparation 40.

These preparations were dried at 110° and analyzed with the following results:

TABLE VII.—COMPOSITION OF ALCOHOL-COAGULA AND OF PROTEOSE PREPARED FROM THE WHEAT EMBRYO.

	Residues of other proteids coagulated by alcohol.			Proteose precipitated by sodium chloride.			Proteose soluble in saturated NaCl sol.			
	31	34	37	32	35	38	33	36	39	40
Carbon	52.36	49.44	51.93	....	49.94	....	48.46	48.70	48.44	48.99
Hydrogen	6.98	6.85	6.87	....	6.80	....	6.70	6.73	6.71	6.85
Nitrogen	16.01	16.00	16.30	16.79	17.08	16.26	16.91	16.76	16.16	16.89
Sulphur	1.85	4.08	1.30	....	1.24	....	27.93	27.81	28.69	1.10
Oxygen	22.80	23.63	23.60	....	24.94	....				26.17
	100.00	100.00	100.00		100.00		100.00	100.00	100.00	100.00
Ash	0.81	14.13	0.95		0.30	0.77	1.13	1.00	0.74	1.27

From these analyses it is seen that the matter insoluble in water, forming preparations **31, 34,** and **37** consists of coagulated proteid apparently mostly derived from leucosin. The high proportion of sulphur in **31** and **34** is due to calcium sulphate, precipitated by alcohol from the aqueous extract.

The remaining preparations have the low percentage of carbon, characteristic of proteoses made by pepsin digestion.

Whether the proteose precipitated by saturating its solution with salt is a different protein substance from that soluble in saturated salt solution or whether difference in solubility is due to the presence of different acid compounds of one and the same protein substance, is not demonstrated, but the agreement shown by these analyses, considering the difficulty of making quite pure preparations, indicates that the latter is the case.

The Proportions of the Various Proteid Substances of the Wheat Embyro.

Twenty grams of fresh germ meal, from which the ether-soluble constituents had *not* been separated, were treated with 500 cc. of water and after shaking for some time, the extract was filtered clear. Two portions, of 100 cc. each, were treated with a few drops of very dilute hydrochloric acid and heated in a boiling water-bath. The coagulum which separated was collected on a filter and its nitrogen determined. To the filtrate from one coagulum, tannin was added and nitrogen was determined both in the precipitate and in the filtrate. Another lot of 20 grams was treated in the same way and nitrogen determined in the heat-coagulum formed in each of two portions of 100 cc. The amount of nitrogen corresponding to one gram of germ meal was found in the four coagula to be, 0.0163 gram, 0.0156 gram, 0.0159 gram, and 0.0162 gram; in the tannin precipitate 0.0062 gram; and in the solution filtered from the latter 0.0062 gram.

Twenty grams of germ meal were extracted with 500 cc. of 3 per cent. sodium chloride solution heated to 70°, whereby the leucosin was coagulated and the globulin and proteose dissolved. Of the clear filtered extract, 100 cc. yielded with tannin a precipitate containing 0.0166 gram nitrogen per gram of meal extracted.

Two portions of the meal, each of one gram, were exhausted with 3 per cent. sodium chloride solution heated to 70°, and nitrogen determined in the residues. The 0.0331 and 0.0309 gram of nitrogen found in the residues were from the leucosin and insoluble nitrogenous bodies, so that the nitrogen belonging to the latter equaled 0.0171 gram and 0.0149 gram. From the average of these figures we find the following amounts of the different forms of nitrogen in one gram of the wheat germ meal.

	Nitrogen. Gram.	
Insoluble in water and salt solution	0.0160	
Insoluble in water but soluble in salt solution	0.0100	= globulin nitrogen.
Soluble in water and coagulable by heat	0.0160	= albumin "
Soluble in water, uncoagulable by heat, precipitable by tannin	0.0050	= proteose "
Not precipitable by tannin.....	0.0060	= non-proteid "
Total	0.0530	
Found by direct nitrogen determination	0.0531	

We have shown that the coagulated leucosin preparations contain about 10 per cent. of nucleic acid, the globulin about 15 per cent., while those of the proteose contain none. Deducting these quantities from the nitrogen given in the table, we find 9.5 per cent. of the embryo to be leucosin, 4.84 per cent. to be globulin, and 3.03 per cent. to be proteose.

The bodies which are represented by the insoluble nitrogen, we have been unable to separate from the embryo. The residue after extraction with hot salt solution contained 0.0076 gram of phosphorus. Since there was in this residue about 0.1000 gram of coagulated leucosin, in which we have usually found about 1 per cent. of phosphorus, we have 0.0061 gram of phosphorus remaining over. In view of the large proportion of nucleic acid found in the extracts of the embryo, it is not improbable that this phosphorus mostly belongs to nucleic acid, in which case there would be about 6.75 per cent. of nucleic acid containing 0.0108 gram of nitrogen, which leaves only 0.0052 gram of nitrogen for proteid matter in the insoluble residue. It seems probable, therefore, that this insoluble nitrogen largely belongs to compounds of the proteid with relatively much nucleic acid.

DIGESTION OF THE PHOSPHORUS-CONTAINING PROTEIDS WITH PEPSIN-HYDROCHLORIC ACID.

Leucosin Nucleate.—Ten grams of the coagulated albumin, preparation 2, were suspended in 400 cc. of water and dissolved by adding 100 cc. of N/10 potassium hydroxide solution. To the nearly clear solution which resulted, an equal volume of 0.4 per cent. hydrochloric acid was added, together with some pepsin, and the mixture digested at 37°. In a short time the solution became perfectly clear, but later deposited a large coherent precipitate, which gradually contracted, but at the same time retained the form of the lower part of the beaker. From this the clear solution was decanted, the precipitate thoroughly washed by decantation, suspended in water and dissolved by adding 28 cc. of N/10 potassium hydroxide solution, an amount of alkali just sufficient to dissolve all the substance and at the same time make the solution neutral to litmus. When to this solution decinormal acid was gradually added, no precipitate appeared until nearly one-half the quantity of acid required for

complete neutralization had been added, but with 28 cc., the solution was neutralized and also completely precipitated, the addition of 2 cc. more acid giving no turbidity in the solution filtered from the precipitate. This precipitate was washed with water and with alcohol and dried over sulphuric acid, forming preparation **41**, weighing 1.54 grams.

To precipitate this substance a quantity of acid was added exceeding that of the alkali employed for solution by just 2 cc. The filtrate from the precipitate, however, required not 2 cc. of alkali, but 8.5 cc. for neutralization to phenolphthalein, showing 6.5 cc. of alkali to have been neutralized by the acid of the nuclein originally dissolved. The neutralized filtrate left, on evaporation, 0.3975 gram of substance, the aqueous solution of which was precipitated by hydrochloric or nitric acid, but *not* by ammonium molybdate solution until after boiling with acid for some little time, when yellow phosphomolybdate was precipitated. These facts indicate the presence in this filtrate of a nucleic acid.

More nuclein was made from the same preparation, **2**, by suspending 30 grams in 0.2 per cent. hydrochloric acid, containing pepsin, which, even at 20°, caused within two hours complete solution of the coagulated proteid. The solution was digested at 37° for forty-eight hours, during which time much nuclein separated, having the appearance and properties of the preparation just described.

After decanting the clear solution and thoroughly washing the residual nuclein, the latter was suspended in water and dissolved in 72 cc. N/10 potassium hydroxide. The solution thus obtained was made neutral to litmus by adding 11 cc. of N/10 hydrochloric acid, but no precipitate appeared till 1.5 cc. more of acid were added. To the solution 72 cc. N/10 hydrochloric acid were added, giving a precipitate which, when washed and dried, made preparation **42** and weighed 3.4 grams. The filtrate from this precipitate, as in the former case, was strongly acid, requiring 12 cc. of N/10 potassium hydroxide to neutralize it to phenolphthalein. Two other preparations of nuclein were made from 8.493 grams of **8**, and 9.804 grams of **11**, both being substances precipitated from the aqueous extract by saturating with sodium chloride. Each portion was suspended in about 300 cc. of 0.2 per cent. hydrochloric acid, containing 0.1 gram of pepsin and,

with frequent stirring, digested at 40° for twenty-four hours. Throughout the digestion a large part of the substance remained undissolved. An equal volume of 0.2 per cent. hydrochloric acid, containing 0.1 gram of pepsin, was again added to each and the digestion continued for twenty-four hours longer. The insoluble matter which remained was not coherent like the two form r nuclein products, but consisted of a white, very finely divided substance which was easily filtered out and washed. After dehydrating with absolute alcohol these preparations were dried over sulphuric acid; from **8**, 4.04 grams of preparation **43** were obtained and from **11**, 4.16 grams of **44**.

Globulin Nucleate.—Fifteen grams of a mixture of nearly equal parts of the globulin preparations **23** and **24** were next suspended in 0.2 per cent. hydrochloric acid, containing 0.2 gram of pepsin, which, within a short time, almost completely dissolved the proteid matter. From this solution, on further digestion, the nuclein separated, forming a coherent deposit. After seventy-two hours' digestion, the clear solution was decanted, the deposit dissolved in a little ammonia and its solution filtered perfectly clear from a very slight gelatinous residue. The resulting solution was then treated with acetic acid, added in excess of the amount necessary to neutralize the solution to litmus. Since, even on standing, the precipitate so produced separated imperfectly, an equal volume of alcohol was added. The substance, which then separated well, was filtered out, washed with dilute and with absolute alcohol and dried over sulphuric acid, giving 2.38 grams of preparation **45**, or about 16 per cent. of the original substance.

The filtrate from the acetic acid precipitate gave a further slight precipitate on adding hydrochloric acid, which had properties characteristic of nucleic acid.

Still another preparation of nuclein was made from the globulin by suspending 10 grams of **27** in water and adding 50 cc. of N/10 potassium hydroxide. This solution was neutralized and an equal volume of 0.4 per cent. hydrochloric acid at once added, producing a turbid solution, which, however, contained no visible particles. To this, pepsin was added and the mixture digested for forty hours, during which time a coherent deposit of nuclein formed on the bottom of the beaker. From this,

the clear solution was decanted. The deposit was then thoroughly washed with water and dissolved in 43 cc. of N/10 potassium hydroxide. To this clear solution 43 cc. of N/10 hydrochloric acid were added, causing a gummy precipitate which could not be filtered until 15 cc. more acid had been added, when the precipitate rapidly settled as a coherent deposit, from which the solution was soon decanted. This solution required for neutralization to litmus 16 cc. N/10 potassium hydroxide, and to phenolphthalein 18 cc. The precipitate, when washed and dried, gave 2.2 grams of preparation **46.**

These six preparations were all dried at 110° and analyzed with the following results:

TABLE VIII.—COMPOSITION OF NUCLEIN FROM THE PROTEIDS OF THE WHEAT EMBRYO.

	41	42	43	44	45	46
Carbon.....	44.87	44.35	42.68	43.35	39.42	41.92
Hydrogen.....	5.82	5.77	5.45	5.47	5.03	5.25
Nitrogen.....	16.04	16.64	16.12	16.01	16.05	17.00
Sulphur	0.97	1.03	0.65	0.85	0.53	0.46
Phosphorus...	4.58	5.07	5.32	4.88	5.27	5.63
Ash.....	0.60	0.78	1.72	1.72	17.42	1.17
P_2O_5 in ash....	0.29	0.55	1.24	0.94	10.56	0.69

If we subtract from the total ash the amount of phosphorus pentoxide found in it, we shall have a determination of the bases contained in the ash of these preparations.

We have calculated these analyses of nuclein free from the bases of the ash and from nucleic acid, in the way previously described, with the following results:

TABLE IX.—COMPOSITION OF PROTEIN MATTER CONTAINED IN THE NUCLEIN.

	41	42	43	44	45	46
Carbon	53.65	54.77	51.80	52.36	52.30	51.64
Hydrogen	7.23	7.46	6.85	6.73	6.91	6.60
Nitrogen	16.68	17.56	16.31	16.31	19.31	18.83
Sulphur	1.98	2.37	1.61	1.89	1.53	1.25
Oxygen.....	20.46	17.84	23.43	22.71	19.95	21.58
	100.00	100.00	100.00	100.00	100.00	100.00

The composition of the proteid matter in **43** and **44** is very nearly that of leucosin except as regards sulphur, the amount of

which is decidedly greater. On the other hand, 41 and 42, which also were derived from preparations whose protein matter was leucosin, differ in composition very decidedly from that substance. This is probably because on pepsin digestion the substance of preparations 43 and 44 remained throughout undissolved, whereas 41 and 42 separated on pepsin digestion from nearly clear solutions and therefore doubtless their protein matter had been to some degree altered by the pepsin before separating as an insoluble compound with nucleic acid. The two nucleins, 45 and 46, from the globulin which also had separated from solution, show similar differences in composition when compared with the unaltered globulin, carbon and nitrogen being higher and sulphur very much higher than in the globulin. The greatly increased proportion of sulphur would indicate that sulphur in some acid form had split from the proteid molecules undergoing hydrolysis and had become a part of the insoluble nuclein, as did the nucleic acid.

CONCLUSION.

The embryo of the wheat kernel contains:

1. A *nucleic acid* in considerable quantity. This acid is insoluble in water, forms soluble as well as insoluble compounds with proteid substances, and on hydrolysis yields guanin, adenin, phosphoric acid, and other products not yet identified. It has the following composition:

NUCLEIC ACID.

Carbon	36.48
Hydrogen	4.48
Nitrogen	16.17
Phosphorus	8.96
Oxygen	33.91
	100.00

This acid is not identical with any nucleic acid heretofore described. On hydrolysis it does not yield any form of sugar. From guanylic acid recently described by Bang it also differs distinctly, in that its potash salt is extremely soluble in cold water and the ratio of phosphorus to nitrogen, being 1 to 4 instead of 1 to 5.

2. *Leucosin*, an albumin (yield about 10 per cent. of the

embryo) formerly found by the writer in small quantity in the whole kernel of wheat, rye, and barley, and abundantly in malt. Leucosin begins to separate as a flocculent coagulum when the very slightly acid aqueous extract of the wheat kernel or wheat embryo is heated to 52°. Even after long heating at 65°, the leucosin is only partly separated, and about one-third more coagulum of the same elementary composition is obtained on raising the temperature from 65° to 100°.

By saturating extracts of the kernel or of the embryo with sodium chloride, the leucosin is largely precipitated from the former as a substance readily soluble again in water, from the latter as an insoluble compound containing about 30 per cent. of nucleic acid. From the latter precipitate, dilute salt solution extracts a small amount of nearly phosphorus-free proteid, which behaves like a globulin, being precipitated by dilution or by dialysis, but having essentially the same ultimate composition as leucosin.

By dialyzing the aqueous extract in water, nearly all the leucosin contained in it is precipitated, not like a globulin, but as an insoluble compound containing about 20 per cent. of nucleic acid. The following figures give the average of accordant analyses, calculated nucleic acid-free, of 18 different preparations representing complete as well as fractional precipitations under the above and other conditions. These figures agree closely with the composition of the leucosin of wheat, rye, barley, and malt.

LEUCOSIN.

Carbon	52.65
Hydrogen	7.04
Nitrogen	16.43
Sulphur	1.32
Oxygen	22.56
	100.00

3. A *globulin*, precipitated in spheroids by dialysis and by dilution as a coherent deposit. The yield is about 5 per cent. of the embryo. The solution of this globulin in 10 per cent. sodium chloride brine becomes turbid on heating to about 87°, and at 90°, on continued heating, a considerable flocculent coagulum separates.

Our preparations of this globulin contained from 6 to 17 per cent. of nucleic acid, most of them from 12 to 15 per cent. From this the proteid could not be separated by fractional precipitation.

Analyses of ten different preparations of this globulin gave very closely agreeing figures when calculated free from nucleic acid, the average of which is as follows:

GLOBULIN.

Carbon	51.57
Hydrogen	7.07
Nitrogen	18.60
Sulphur	0.65
Oxygen	22.11
	100.00

In composition and properties this globulin agrees with that found by the writer in the kernels of wheat, rye, and barley. So far as we have been able to observe, it differs from edestin, the crystalline globulin obtained from seeds of hemp, flax, and squash only in containing two-thirds as much sulphur.

4. *Proteose*, precipitated by saturating the aqueous extract, freed from globulin and albumin, with salt. One preparation, 35, was phosphorus-free, and had the following composition:

PROTEOSE.

Carbon	49.94
Hydrogen	6.80
Nitrogen	17.08
Sulphur	1.24
Oxygen	24.94
	100.00

5. *Proteose*, soluble in the salt-saturated solution filtered from the foregoing proteose and obtained free from phosphorus by precipitating with alcohol its solution freed from salt by dialysis. The average of analyses of four preparations of this proteose is the following:

PROTEOSE.

Carbon	48.65
Hydrogen	6.75
Nitrogen	16.68
Sulphur	1.10
Oxygen	26.82
	100.00

These proteoses together form about 3 per cent. of the embryo.

6. About one-third of the total nitrogen of the embryo is not extracted by water and salt solutions and appears to belong to insoluble compounds. This nitrogen is accompanied by phosphorus corresponding to about 6.75 per cent. of nucleic acid, which would contain two-thirds of this insoluble nitrogen. It seems probable, therefore, that this insoluble nitrogen belongs largely to insoluble compounds of nucleic acid and protein.

7. These phosphorus-containing preparations of globulin and leucosin, when digested with pepsin-hydrochloric acid, yield nuclein in proportion to the phosphorus which they contain. Calculated free from nucleic acid, the analyses of these nucleins show the protein constituent to have nearly the same composition as the proteid from which they were derived, the most marked difference being a greater proportion of sulphur in the former.

8. The proteids of the embryo differ from those of the dormant endosperm, of this as well as of other seeds, in the facility with which they undergo changes. These changes are the result of a redistribution of acids among the protein and other basic molecules, so that compounds form in the extracts of the embryo which contain various proportions of nucleic acid according to the changing conditions.

The writer has shown that the globulin, edestin, forms crystalline compounds with one and with two molecules of acid and also compounds with a greater number of acid molecules. There is reason to believe that all other native protein substances form similar compounds; in other words, that proteins are distinctly polyacid bases and that the acid characters which proteids display are due to acids united to their protein molecules probably in the same manner as in the salts of the purin bases.

These nucleic acid compounds of the protein constituents of the wheat embryo appear to be compounds of this order. According to this view, no special distinction can be made between nucleins and nucleoproteids, the former being simply compounds containing a greater number of molecules of nucleic acid united to one molecule of protein.

That the wheat embryo in fact contained the same nucleic acid *compounds* as we have obtained from the extracts, is highly

improbable. All that we can conclude is that the embryo contains the different protein substances described, together with nucleic acid, and that these may unite to form a number of different compounds according to the conditions which prevail at any given time.

THE PROTEIDS OF THE EGG YOLK.[1]

By Thomas B. Osborne and George F. Campbell.

Received June 16, 1900.

The yolks of a large number of freshly laid hens' eggs were broken up by straining through a sieve and mixed with an equal volume of saturated pure sodium chloride brine. A somewhat turbid solution resulted, which was shaken out with about one-third its volume of ether, containing a little alcohol. After standing one night, a clear reddish ethereal layer separated, leaving the pale yellow aqueous solution almost clear. After shaking out a second time with ether the aqueous solution was dialyzed for forty-eight hours, whereupon a large quantity of proteid separated in spheroids which united to a salvy mass. This was freed, as far as possible, from the liquid, by draining on filters, redissolved in 10 per cent. brine and its solution dialyzed for three days. From the semisolid mass thus obtained the liquid was decanted and the precipitate dissolved in 10 per cent brine. A little transparent gummy substance (lecithin?) remained undissolved which rendered filtration very difficult. By filtering under considerable pressure on a thick layer of paper pulp about 700 cc. of *perfectly clear* filtrate, *a*, and 2,000 cc. of very nearly clear filtrate, *b*, were obtained. The latter, *b*, was dialyzed for four days, when the large deposit was filtered out and treated with about a liter of salt solution. A gelatinous, almost pasty mixture resulted, which was shaken out with ether. The two liquids at once separated, the ethereal being clear and strongly yellow in color and the aqueous almost perfectly clear and not at all gummy. Shaken out three times more with ether, this aqueous solution became suddenly opaque and gelatinous. The mass was dialyzed free from chlorides, the solid deposit was washed with alcohol and with ether and dried over sulphuric

[1] From advance sheets of the report of the Connecticut Agricultural Experiment Station for 1899, communicated by the authors.

acid, giving preparation 1, weighing 60 grams. The *clear* solution, *a* (700 cc.), was diluted with three volumes of distilled water, and allowed to stand over night at a temperature of 6°. The proteid, which had separated on dilution, soon settled as a clear, transparent layer, from which the clear solution, *c*, was decanted completely. The proteid was readily and completely soluble in 10 per cent. brine to a perfectly clear pale yellow solution, which, when dialyzed until wholly free from chlorides, yielded the proteid in spheroids that subsequently united to a semisolid mass. The latter was filtered out, washed thoroughly with water and with alcohol, and dried over sulphuric acid, giving 32.1 grams of preparation 2. The solution, *c* (decanted from the precipitate produced by dilution that yielded 2), was treated with 1,000 cc. more water. This caused a precipitate which within two hours formed a semifluid deposit. From this the solution, *d*, was decanted, and the deposit dissolved in brine yielding a perfectly clear solution, which was dialyzed until free from chlorides.

The proteid thus precipitated was filtered out, washed with absolute alcohol as long as anything could be removed thereby, and then dried over sulphuric acid, giving preparation 3, weighing 16.5 grams.

The solution, *d*, was dialyzed free from chlorides, the precipitated spheroids were filtered out, washed with absolute alcohol and dried over sulphuric acid, making 5 grams of preparation 4.

The solution, page 413, filtered after forty-eight hours' dialysis from the substance that yielded the foregoing preparations, was further dialyzed until almost all its dissolved proteid had separated.

The precipitate thus produced was filtered out, dissolved in brine and the solution shaken out three times with ether. On shaking out the third time, the solution suddenly changed to an opaque jelly. This was then placed in a dialyzer, and when all the salt had been removed, the insoluble proteid was washed with absolute alcohol until everything soluble therein was extracted. Dried over sulphuric acid, this preparation, 5, weighed 75 grams. All these preparations were dried at 110° and analyzed with the following result:

	1	2	3	4	5
Carbon	50.82	51.21	51.10	50.69	50.48
Hydrogen	7.11	7.07	7.21	7.06	7.11
Nitrogen	16.04	16.11	16.23	16.40	15.50
Sulphur	1.11	1.05	1.00	1.05	1.02
Phosphorus	0.91	0.81	0.79	1.20	0.96
Ash	2.37	2.49	1.56	3.23	3.19
P_2O_5 in ash	1.65	1.79	1.14	2.30	2.15

The ash of all these preparations consisted of sodium metaphosphate containing about 70 per cent. of P_2O_5. We have therefore subtracted from the total ash its phosphorus pentoxide, the remainder representing the ash to be deducted in calculating the percentage composition of the proteid substance.

Any chlorine or sulphur that might belong to the ash would necessarily be lost during incineration.

Calculating these preparations free from ash, as thus corrected, we have:

	1	2	3	4	5
Carbon	51.18	51.56	51.31	51.16	51.00
Hydrogen	7.14	7.12	7.24	7.12	7.18
Nitrogen	16.16	16.23	16.30	16.55	15.66
Sulphur	1.12	1.06	1.00	1.06	1.03
Phosphorus	0.92	0.82	0.79	1.21	0.97
Oxygen	23.48	23.21	23.36	22.90	24.16
	100.00	100.00	100.00	100.00	100.00

1 and 5 represent the two main fractions that weighed 60 and 75 grams respectively; while 2, 3, and 4 represent fractions of 1, whose weights were respectively 32, 16.5, and 5 grams.

In composition all are nearly alike. A little more phosphorus was found in 4 than in the other preparations, probably because a larger proportion of some phosphorus-containing acid was combined with the protein of this final, very soluble fraction, which formed less than 10 per cent. of the total vitellin.

We have not yet succeeded in preparing this phosphorized acid free from proteid. The body, which we have thus prepared from egg yolk and analyzed, is not present, as such, in the egg, for the proteid substances of the yolk are readily and wholly soluble in salt solution, whereas all these preparations are entirely insoluble in salt solution. Insolubility in the cases of 2, 3, and 4 was caused by washing with alcohol, which at the

same time removed much lecithin. Lecithin was not present as an admixture, but was chemically combined with the proteid, forming a compound soluble in saline solutions and having the properties of globulin, as is shown by the following experiments.

A. The alcoholic washings from the three successive fractions, **2, 3,** and **4,** were evaporated and left residues of crude lecithin weighing 6.4796, 3.5913, and 1.3150 grams, respectively. Adding these quantities to the weights of the corresponding fractions dried at 110°, we have the amounts of lecithin-protein compound originally composing them. These contained 18.00, 19.4, and 22.23 per cent. of lecithin, respectively.

B. The yolks of two eggs were directly extracted with ether until practically nothing more was removed. The residual matter was then, as far as possible, dissolved in 10 per cent. salt solution, filtered perfectly clear and the solution diluted with water until an abundant precipitate separated. This was filtered out, dissolved in salt solution, and filtered perfectly clear. This solution and that filtered from the precipitate previously produced by diluting with water were separately dialyzed.

The proteid precipitates thus obtained were filtered out and washed with water and alcohol. The part precipitated by dilution was found to contain 17.5 per cent. of lecithin, that which remained in the diluted brine, 22.3 per cent.,—results in pretty close accord with those already stated.

C. Part of a large quantity of yolk vitellin which had suddenly become insoluble on shaking with ether, was thoroughly washed with water and then completely extracted with alcohol until all the lecithin, equal to 13.31 per cent. was removed. The solution, from which the above large quantity of insoluble proteid had originally separated, still contained a little protein matter and was therefore saturated with ammonium sulphate and the salt solution of the precipitate so produced was dialyzed. The substance which then separated in spheroids, after thorough washing with water, was still readily soluble in salt solution, but when washed with alcohol became insoluble and yielded to the alcohol 24.2 per cent. of lecithin.

Hoppe-Seyler considered this lecithin to be chemically combined with the proteid, with which view our experience is in full harmony. It is not possible that such large quantities of

lecithin are simply admixed with the protein matter, for were this the case it could be readily removed by ether. Furthermore we could not dissolve a mixture of globulin and lecithin in brine and obtain a clear solution easy to filter. That the proteid should unite with lecithin is to be expected, since protein readily combines with acids. We must, accordingly, consider the protein of egg yolk to be largely, if not wholly, a lecithin compound which dissolves in salt solution, and behaves like a globulin.

Saline extracts of egg yolk, like those of plant-seeds, contain, according to circumstances, mixtures of compounds of the protein molecule with several different numbers of molecules of lecithin, of which the more soluble compounds contain the larger number of acid molecules.

That we find such a large proportion of lecithin in these compounds is accounted for by its great molecular weight. If the molecular weight of the protein were 15,000,[1] its compound with four molecules of lecithin would contain over 17 per cent. of the latter.

Although we are not yet in a position to distinguish between these several compounds, it is nevertheless now necessary to make a distinction between the vitellin as it exists in the yolk, combined with lecithin, and the insoluble substance free from lecithin, which we have prepared and analyzed. As the designation vitellin has generally, if not always, been understood to apply to a protein substance, we suggest that this term be henceforth reserved for the protein, which in egg-yolk is united to lecithin and not to the compounds formed by their union, which may more properly be called lecithin-vitellin.

These considerations raise the question, are the preparations analyzed, vitellin, as defined above, or are they compounds of this protein with some other, at present unknown substance. Since the preparations yield paranuclein on digestion with pepsin, it appears highly probable that they contain paranucleic acid, but in less proportion than occurs in paranuclein.

To test this hypothesis we treated 10 grams preparation 5 with 100 cc. of N/10 potassium hydroxide solution, and after

[1] We have pointed out in a former paper (This Journal, **21**, 486), the reasons which make it probable that the weight of the protein molecule may be about 15,000.

standing some time added enough 0.4 per cent. hydrochloric acid to neutralize the alkali and give an excess of acid equal to 0.2 per cent. of the solution. Pepsin was then intermixed and the solution digested at 40° for forty hours. After some time a voluminous precipitate separated from the nearly clear liquid. This was filtered out, washed thoroughly with water, and mixed with 45 cc. N/10 potassium hydroxide solution, which dissolved the precipitate and just neutralized its acid reaction to phenolphthalein. This solution was filtered perfectly clear and 45 cc. of N/10 hydrochloric acid were added, which threw out the paranuclein as a voluminous gelatinous precipitate. This was filtered out, washed thoroughly with water and with alcohol, and dried over sulphuric acid. This preparation, 6, weighed 2.38 grams. Another preparation of paranuclein was made by suspending 50 grams of preparation 5 in 0.2 per cent. hydrochloric acid, adding pepsin and, after digesting for twenty-four hours, adding more acid and pepsin and continuing the digestion twenty-four hours longer. The insoluble paranuclein was then filtered out, washed thoroughly with water and with alcohol, and dried over sulphuric acid. It weighed 15.7 grams. Preparation 7.

The yolks of 120 eggs were mixed with an equal volume of saturated sodium chloride brine and the mixture was shaken with ether containing a little alcohol. A perfectly clear red-yellow ether-layer and a clear pale yellow aqueous layer soon separated. The ether was drawn off and the aqueous solution again shaken with ether, which caused a part of the proteid to become insoluble, rendering the solution opaque and gelatinous. After the ether had separated, the aqueous solution was dialyzed until free from chlorides, when it was filtered and the very voluminous precipitate was suspended in 4 liters of 0.2 per cent. hydrochloric acid and digested with 3 grams of pepsin. After twenty-four hours the solution was decanted from a large deposit of insoluble matter; the latter was mixed with about one-third its volume of 0.4 per cent. hydrochloric acid and the digestion continued forty-eight hours longer, whereby the amount of insoluble matter was much reduced. The latter was then filtered out and repeatedly extracted with alcohol until the evaporated alcoholic washings left no residue. This required a

large quantity of alcohol and more than a week of time. The residue, air dried, weighed 32 grams and formed preparation 8.

These preparations were then dried at 110° and analyzed, with results as follows:

COMPOSITION OF PARANUCLEIN.

	6	7	8
Carbon	46.69	47.72	44.48
Hydrogen	6.77	6.80	6.52
Nitrogen	14.66	14.64	14.34
Sulphur	0.86	0.94	0.82
Phosphorus	3.29	2.52	4.19
Ash	1.89	5.01	3.43
P_2O_5 in ash	0.83	2.47	1.61

Assuming that the yolk proteid and the paranuclein are both compounds of paranucleic acid, we should be able to calculate the composition of the protein substance in our preparations if we knew the composition of this acid. Unfortunately we do not know this acid in the free state.

Liebermann and others have thought that paranucleic acid is identical with metaphosphoric acid, but we think it extremely improbable that metaphosphoric acid can exist in animal tissues.

In seeking to harmonize the analyses of paranuclein with those of the paranucleoproteid we find that they are brought into more or less accordance by reckoning them free from ash and from certain phosphoric acid radicals. Assuming that the hydrogen of the phosphoric acid is replaced by protein, we have "corrected" our analyses for the following phosphoric acids; *viz.*, HPO_3, H_3PO_4, $H_4P_2O_9$, and H_5PO_5 by subtracting from the ash its P_2O_5 and to the remainder (the bases) adding the phosphorus and oxygen of the respective acids and, after deducting their sum from 100, recalculating these remainders to percentage statements.

Corrected for PO_3 we find no satisfactory agreement, as is shown by the following figures obtained by thus correcting the analyses of preparations 2 and 6.

	2	6
Carbon	52.68	50.86
Hydrogen	7.28	7.37
Nitrogen	16.57	15.97
Sulphur	1.08	0.94
Oxygen	22.39	24.86
	100.00	100.00

Correcting for the other three acids, however, we find a close agreement between all the analyses of the preceding preparations as is seen in the following tables:

COMPOSITION OF THE PARANUCLEOPROTEID AND THE PARANUCLEIN CALCULATED FREE FROM PO_4 AND ASH.

	Paranucleoproteid.					Paranuclein.		
	1	2	3	4	5	6	7	8
Carbon....	52.67	52.89	52.59	53.14	52.57	52.54	53.17	52.12
Hydrogen.	7.37	7.30	7.42	7.40	7.40	7.60	7.57	7.64
Nitrogen..	16.62	16.64	16.70	17.19	16.11	16.50	16.31	16.80
Sulphur...	1.15	1.09	1.03	1.10	1.06	0.96	1.05	0.96
Oxygen...	22.19	22.08	22.26	21.17	22.86	22.40	21.90	22.48
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The agreement of these figures makes it plain that the paranuclein consists of one or more compounds of the protein (vitellin) containing a larger proportion of the same acid that exists in the paranucleoproteid from which it originated. These protein compounds with paranucleic acid are therefore analogous to those with the nucleic acid which we have obtained from the wheat embryo and described in the preceding paper.

If, as many seem to think, the paranucleic acid is not in fact H_3PO_4, it may be found to be either $H_8P_2O_9$ or H_5PO_5. These acids differ in composition so little from H_3PO_4 that we find nearly or quite as close agreement when the analyses are calculated free from either of them, as the following table shows, in which is given the result of calculating the analyses free from P_2O_9 and ash.

COMPOSITION OF THE PARANUCLEOPROTEID AND THE PARANUCLEIN CALCULATED FREE FROM P_2O_9 AND ASH.

	Paranucleoproteid.					Paranuclein.		
	1	2	3	4	5	6	7	8
Carbon	52.79	53.00	52.70	53.31	52.71	53.05	53.56	52.78
Hydrogen.....	7.39	7.32	7.44	7.42	7.42	7.69	7.63	7.74
Nitrogen.....	16.66	16.67	16.74	17.25	16.19	16.66	16.43	17.02
Sulphur	1.15	1.08	1.03	1.10	1.06	0.98	1.05	0.97
Oxygen.....	22.01	21.93	22.09	20.90	22.62	21.62	21.33	21.49
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Considering the great differences in phosphorus-content of these preparations, the striking agreement of the analyses thus

calculated makes probable that the acid combined with the protein has nearly the composition H_3PO_4 and contains little, if any, organic matter, or else contains an organic radical, whose composition is very nearly like that of the protein itself.

It may possibly be a methyl or ethyl phosphoric acid or some other quite simple organic phosphoric acid, but probably not so complex an acid as glycerophosphoric, as we have found a wide difference between the analyses when corrected for this acid.

It seems probable in view of these facts that further study will lead to the isolation and positive identification of this acid.

CONCLUSION.

Sodium chloride solutions dissolve from egg yolk a large amount of protein matter which has the properties of a globulin, being precipitated by diluting or dialyzing its solutions.

The substance soluble in salt solution consists of a mixture of compounds of protein matter with lecithin. Preparations of these compounds contain from 15 to 30 per cent. of lecithin. The more soluble products obtained by fractional precipitation contain larger proportions of lecithin than the less soluble; that is, they are more acid compounds. These compounds might well be called lecithin-nucleovitellin.

The lecithin thus combined is not removed by ether, but readily by alcohol. The insoluble lecithin-free proteid, obtained by treating the lecithin compounds with alcohol, has a constant composition when obtained from successive fractional precipitations of the lecithin compound. The following is the average of five accordant analyses representing fractional precipitations of the substance :

COMPOSITION OF NUCLEOVITELLIN.

Carbon	51.24
Hydrogen	7.16
Nitrogen	16.38
Sulphur	1.04
Phosphorus	0.94
Oxygen	23.24
	100.00

This substance on digesting with pepsin yields paranuclein of variable composition. When the analyses of the nucleovitellin

and the paranuclein are calculated free from phosphoric acid, H_3PO_4, possibly identical with "paranucleic acid", the composition found for the organic part of all of these preparations is so nearly the same as to show that the proteid and the nuclein are both compounds of one and the same proteid body, vitellin, with a phosphoric acid, possibly H_3PO_4, $H_5P_2O_9$, or some very simple organo-phosphoric acid. The composition of the organic part of the preparations calculated free from H_3PO_4 gives as the average for eight preparations of the paranucleoproteid and the paranuclein, the following figures:

COMPOSITION OF VITELLIN.

Carbon	52.71
Hydrogen	7.46
Nitrogen	16.64
Sulphur	1.05
Oxygen	22.14
	100.00

THE PROTEIN CONSTITUENTS OF EGG WHITE.[1]

BY THOMAS B. OSBORNE AND GEORGE F. CAMPBELL.

Received June 16, 1900.

A RECENT paper by the writer[2] gave an account of preparations of crystallized egg albumin which justified the conclusion that with the substance commonly called ovalbumin there is associated one or more other protein bodies, the properties of which were not definitely ascertained.

We have since repeated this work on a larger scale and have not only confirmed the former observations, but have obtained much additional information respecting these other protein bodies.

FRACTIONAL PRECIPITATION OF EGG WHITE.

Six liters of the whites of freshly laid eggs were gradually and carefully mixed with an equal volume of saturated ammonium sulphate solution and formed the precipitate, A, which was filtered off.

To the filtrate saturated ammonium sulphate solution was

[1] From advance sheets of the report of the Connecticut Agricultural Experiment Station for 1899, communicated by the authors.

[2] Report Conn. Agr. Exp. Station for 1898, and this Journal, **21**, 486.

added until a small permanent precipitate was produced, and then a mixture of 600 cc. of saturated ammonium sulphate solution, 830 cc. of water, and 27 cc. of concentrated hydrochloric acid were gradually added. After standing over night, the large quantity of fine needle-shaped crystals which separated, fraction B, was filtered out.

The filtrate, mixed with saturated ammonium sulphate solution until a small precipitate again formed, was allowed to stand over night, during which time another crystalline precipitate, C, separated.

By treating the filtrate from C in the same manner, another precipitate, D, was obtained, which consisted wholly of spheroids.

All the protein matter which remained in the filtrate from D was precipitated by saturating the solution with ammonium sulphate, and this made preparation E. These several fractions were then further divided as follows:

Precipitate A was treated with water, strained with difficulty, owing to its gummy character, through bolting-cloth, and the slightly turbid solution thus obtained was dialyzed for several days. The considerable mucin-like precipitate which separated was filtered out, washed with much water, and dehydrated with absolute alcohol, giving 26.88 grams of preparation A.1.a.

The filtrate from the precipitate of A.1.a., saturated with ammonium sulphate, yielded a precipitate which was treated with a little water, and the part insoluble therein filtered out; the filtrate was allowed to evaporate until an amorphous precipitate separated. This precipitate was filtered out, united with the insoluble part of the ammonium sulphate precipitate, dissolved in water, and its solution dialyzed, yielding a precipitate which resembled that previously obtained by dialysis, being largely composed of gummy, mucin-like clots. This, when well washed with water and alcohol and dried, gave 7.28 grams of A.1.b. The dialyzed solution from which A.1.b had separated, when treated with ammonium sulphate, yielded a precipitate which was filtered out, pressed on filter-paper, dissolved in water and this solution dialyzed free from sulphate. This dialyzed solution, evaporated at 40°, left a residue, marked A.2, weighing, dry, 16 grams.

The solution containing sulphate of ammonium, from which had been separated the substance yielding the two last-named preparations, was further concentrated by gradual evaporation, and yielded a mass of needle-shaped crystals. These were separated by filtration, dissolved in water, the solution was dialyzed till free from ammonium sulphate and evaporated at 40°, giving preparation A.3, weighing 11.56 grams.

Precipitate B.—This, consisting wholly of well-formed crystals, was dissolved in water and treated with saturated ammonium sulphate solution, which was added until precipitation began. After standing over night, the wholly crystalline precipitate was filtered out and the filtrate, marked b.2, treated as will be described later. The precipitate was again dissolved in water and enough ammonium sulphate added to give a precipitate from which, after a time, the solution was decanted. After pressing out the mother-liquor, the precipitate was dissolved in water and the clear solution dialyzed until wholly free from sulphate, when it was evaporated at 50°, giving 36 grams of B.1.

The filtrate from the precipitation of B.1 treated with more ammonium sulphate, on standing twelve hours, deposited a large crop of finely developed crystals. This was filtered out and, treated in the manner described for B.1, gave 59 grams of B.2. The filtrate from B.2 was added to solution b.2.

Precipitate C.—The filtrates from B.1 and B.2, forming solution b.2, were united with the aqueous solution of fraction C, ammonium sulphate was added to incipient precipitation and the mixture was allowed to stand over night. The substance, which separated in spheroids, was filtered out, dissolved in water and recrystallized by adding ammonium sulphate. On standing, the substance deposited in large aggregates of crystals, extending from the bottom of the dish in warty masses, some of them more than 2 cm. long and 0.5 cm. in diameter. These masses were wholly composed of exceptionally large and well-formed needle crystals. After separating from the mother-liquor, this crystalline mass was dissolved in water and the solution dialyzed till free from sulphate, and, evaporated at 50°, gave 45 grams of C.1.

The filtrate from C.1, on standing, yielded a very large quantity of substance which was composed almost wholly of crystals, only a very few spheroids being detected among them. This

precipitate, by the treatment applied to C.1, yielded 106 grams of C.2.

The filtrate from the first precipitation of C.1 was treated with more ammonium sulphate, the filtrate from C.2 was added, and the mixture allowed to evaporate slowly until a precipitate separated, which consisted of a mixture of crystals and spheroids. This yielded C.3, weighing 32.5 grams.

Precipitate D.—This was dissolved in water, ammonium sulphate solution was added and the mixture allowed to stand until a considerable separation of spheroids occurred, which, on settling, formed a clear, transparent deposit on the bottom of the dish.

After several reprecipitations, always without obtaining crystals, and thinking that the absence of crystals might be due to a deficiency of acid, we added to the solution 3 cc. of concentrated hydrochloric acid mixed with much ammonium sulphate solution. On standing, however, this solution, as before, deposited only spheroids. The deposit, after decanting the solution, was dissolved in water and its solution, after adding ammonium sulphate to incipient precipitation, was allowed to evaporate slowly. When considerable deposit had formed, the solution was decanted and allowed to concentrate until nearly all the remaining proteid matter had formed a coherent deposit of spheroids. These two deposits were each separately dissolved in water, their solutions dialyzed free from ammonium sulphate and evaporated to dryness at 50°, thus giving D.1, weighing 30 grams and D.2, weighing 20 grams.

The solution, decanted from the first precipitate which separated after adding acid, gave on evaporation a precipitate consisting wholly of spheroids, which formed D.3, weighing 55 grams. The solution from this, on further concentration, deposited nearly all the dissolved protein in the form of spheroids, which, by the usual treatment, gave 10 grams of D.4.

Precipitate E.—This, when dissolved in water, yielded a brilliant yellow solution, slightly acid to litmus. Thereto saturated ammonium sulphate solution, containing 1 cc. of concentrated hydrochloric acid, was added, which, on standing, caused a precipitate. This was filtered off, dissolved in water, ammonium sulphate was added to incipient precipitation, and after standing

seven days, the deposit of spheroids which had separated was filtered out, yielding 6.2 grams of E.1. The filtrate contained no protein matter.

The filtrate from the first reprecipitation of E was mixed with ammonium sulphate until a considerable amorphous precipitate had formed. This was filtered out, dissolved in water, and after adding ammonium sulphate and allowing the solution to stand six days, it gave a precipitate of spheroids which yielded E.2, weighing 15.65 grams.

The solution decanted from the first precipitation of E.2, after evaporating for six days, gave a deposit of spheroids mixed with a little amorphous matter, which was marked E.3, and weighed 22.5 grams.

We thus obtained from 6 liters of egg white, seventeen different fractions, containing 504 grams of substance, 262 grams of which were wholly crystalline, and since at least one-half of C.3 consisted of crystals, the total amount of crystalline matter was 278 grams. Evidently more than one-half the protein constituents of egg white can be crystallized. The yield of crystallized albumin was about the same as that obtained by the writer in his previous investigation, being about 4.4 grams of crystallized albumin per 100 cc. of egg white, as against 5.3 and 4.9 grams previously obtained.

Fractions E.2 and E.3.—Since the fractions E.2 and E.3 appeared, by their behavior on heating with water, to contain at least three protein substances, the greater part of each preparation was treated with water. E.2 contained some matter insoluble in water which could not be filtered out, while E.3 gave a clear solution. Both solutions, heated in a water-bath, gave a flocculent coagulum at 58° which, even after heating at 65° for some time, could not be filtered out. The temperature was therefore raised to 72° and held there some time, which caused more coagulum to separate in both solutions, but as the solutions could not even then be filtered, the bath was heated to boiling. With the rising temperature the coagulum rapidly increased, so that it could soon be filtered out on cloth. After thoroughly washing each coagulum with hot water and alcohol and drying over sulphuric acid, we obtained preparations E.2.a, weighing 6 grams, and E.3.a, weighing 8.6 grams. The clear, bright,

yellow filtrates from these were united and poured into several volumes of alcohol, which threw out a white precipitate and held the yellow coloring-matter in solution.

The precipitate, E.4, washed with alcohol and ether and dried over sulphuric acid, weighed 5 grams.

These preparations were then dried to constant weight at 110° and analyzed.

The heat coagulation points of their solutions, containing 2.5 per cent. of the protein substance and 10 per cent. of sodium chloride, were determined by gradually heating in a water-bath. In Table I, page 428, T indicates the temperature at which the solution first became turbid, and F that at which flocks separated.

The specific rotation was determined with a Schmidt and Haensch polarimeter, the readings on the sugar scale being converted into degrees of circular polarization by multiplying by 0.346.

In the table the percentages of nitrogen marked K, were found by the Kjeldahl process, those marked A, by the Dumas or absolute method.

These results entirely confirm those given in our former paper, but in consequence of the more extended series of fractional precipitations in the work now under discussion we have plainer evidence of the presence of the several protein constituents of the egg white.

Of these fractions, B.1, B.2, C.1, and C.2 consist of ovalbumin, separated in a completely crystalline condition, and, with the exception of a trace present in C.2, are wholly free from the lower coagulating albumin, thus demonstrating this latter to be a distinct substance. The rotation and elementary composition of these fractions are essentially the same as that given in our former paper for similar products.

In fraction E.4 we have ovomucoid not coagulated by heat, with a specific rotation of 61.5° and of the same composition as Möner[1] and Zanetti[2] found for this substance. The successive fractions, from C.3 to E.3.a inclusive, all have a higher specific rotation and contain a relatively considerable amount of an albumin coagulating at a much lower temperature than ovalbumin.

[1] *Ztschr. physiol. Chem.*, **18**, 525.

[2] *Ann. di Chim. e Farmac.*, No. 12, 1897.

TABLE I.—THE WEIGHT, ROTATION, COMPOSITION, AND HEAT COAGULATION TEMPERATURES OF THE FRACTIONAL PRECIPITATES OF EGG WHITE.

Preparation.	Weight Grams.	$[\alpha]_D$.	Calculated ash-free.						Ash.	Coagulation temperature.	Remarks.
			C.	H.	N.	S.	P.	O.			
A.1.a.	26.88	−21° 9′	50.69	6.71	14.49	2.280	trace	25.830	0.53	T 70° F 75°	Coagulum nearly disappears on boiling and reappears on cooling.
A.1.b.	7.28	. . .	50.95	6.85	14.82	1.938	none	25.442	1.41	. . .	
A.2.	16.00	−31° 50′	52.76	7.12	15.48K 15.58K	1.687	0.123	22.770	0.41	T 64° F 70°	Practically all coagulated above 70°.
A.3.	11.56	. . .	52.40	7.12	15.77K 15.66K	1.729	0.002	23.02	0.28	T 66° F 70°	
B.1.	36.00	−29° 53′	52.59	7.10	15.59K 15.51K	1.613	0.126	23.021	0.136	T 70° F 71°	None coagulated below 70°.
B.2.	59.00	−29° 13′	52.78	7.09	15.54K 15.60A	1.619	0.127	22.814	0.150	T 68° F 70°	None coagulated below 68°.
C.1.	45.00	−30° 2′	52.75	7.31	15.57A 15.43K	1.613	0.131	22.696	0.350	T 69° F 71°	None coagulated below 69°.
C.2.	106.00	−30° 48′	52.79	7.00	15.57K 15.63A	1.634	0.126	22.850	0.20	T 62° F 65°	Only a trace coagulated at this temperature. The rest separated at 70° and above.
C.3.	32.40	−34° 2′	52.24	7.08	15.55A	1.755	0.085	23.290	0.290	T 57° F 57°	Considerable coagulated below 70°, but most above 70°.
D.1.	30.00	−34° 18′	52.41	6.93	15.57A	1.796	0.106	23.188	0.14	T 51° F 53°	Much coagulated below 70°, but most above 70°.
D.2.	20.00	−37° 14′	52.29	6.94	15.61A	1.886	0.062	23.212	0.04		
D.3.	55.00	−41° 22′	51.62	6.94	15.23A 15.27K	1.972	0.048	24.170	0.10	T 52° F 55°	About 50 per cent. coagulated below 60°, about 35 per cent. at 70° and above. The rest did not coagulate.
D.4.	10.00	−51° 30′	50.56	6.80	13.64A 13.54A	1.997 2.017	trace	27.033	0.11	T 62° F 64°	Aqueous solution on heating, formed a clear jelly. Salt solution coagulated at 64°, and above. Much uncoagulated.
E.1.	6.20	−33° 36′	51.91	6.94	15.02A 15.27K	1.700 1.710	trace	24.285	0.49	T 59° F 60°	Probably about 40 per cent. was coagulated below 70°, much above 70°.
E.2.	15.65	−43° 7′	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	Less appeared to coagulate below 70° than of E.1 when heated in water with no salt.
E.3.	22.50	−49° 5′	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	Less separated below 70° than of E.2 when heated in water.
E.2.a.	6.00	. . .	52.40	6.91	15.69A	1.780	0.090	23.131	c.66	. . .	Not much separated below 70° when heated in water.
E.3.a.	8.60	. . .	51.94	6.84	15.31A	1.629	0.045	24.236	0.36	. . .	Not much separated below 70° when heated in water.
E.4.	5.00	−61° 38′	49.02	6.45	12.71A	2.379	0.11	29.441	0.750	. . .	No coagulum.

The fractions C.3, D.1, and D.2 have nearly the same rotation temperatures of coagulation and composition. We therefore subjected these to fractional precipitation, in order to determine whether they were mixtures of different proteids or were essentially one substance. The greater parts of these preparations were accordingly united, dissolved in about 400 cc. of water and saturated ammonium sulphate solution added, until a decided precipitate had formed. This was filtered out, freed from mother-liquor, dissolved in water, the solution dialyzed until free from ammonium sulphate and then evaporated to dryness below 50°. The residue, F.1, weighed 7.5 grams. The filtrate from F.1, on evaporating at the room temperature, deposited a quantity of spheroids, mixed with a few crystals of ovalbumin. This deposit was filtered out, dissolved in water, to the solution saturated ammonium sulphate solution was added until incipient precipitation set in, and the whole was allowed to stand until a considerable precipitate composed of spheroids mixed with a few crystals had formed. This was filtered out and, in order, if possible, to convert the whole into crystals, the original reaction of the egg white was imitated by making the aqueous solution of the precipitate slightly alkaline with ammonia. A very small excess of hydrochloric acid was added and then ammonium sulphate to incipient precipitation, thus reproducing, as closely as we could, the conditions under which the first crystalline separation had been obtained. After standing, a precipitate formed which contained no crystals. This was filtered out and, by the usual treatment, gave F.2, weighing 18.32 grams. The solutions filtered from the two preceding precipitations of F.2 were saturated with ammonium sulphate, and the resulting precipitate dissolved in water; its solution, dialyzed and evaporated below 50°, gave F.2.a, weighing 7.83 grams. The filtrate from the first precipitation of F.2 on further evaporation gave a precipitate of spheroids which was dissolved in water, and by the treatment employed for separating F.2.a, gave 16.7 grams of F.3.

The solution filtered from the second precipitation of F.3 was saturated with ammonium sulphate and from the precipitate produced by the usual treatment, F.3.a, weighing 5.38 grams, was obtained. These several preparations were found to have the

rotation, heat-coagulation temperatures and compositions given in Table II.

While none of these fractions was obtained free from the lower coagulating albumin, the relative proportions of the two albumins which the different fractions contained varied greatly, which fact, in conjunction with their rotation and composition, shows that a decided, though incomplete, separation has been effected.

In order to separate more completely the proteids which these mixed fractions appeared to contain, we dissolved 13.4 grams of D.3 in 500 cc. of 10 per cent. brine, and heated the filtered and perfectly clear solution slowly in a large water-bath. A flocculent coagulum began to separate at 52°, which, after heating for some time at 60°, was filtered off. The filtrate was then removed and this coagulum was washed with pure water, which, after the salt was removed, dissolved the coagulum almost completely. From these washings the proteid was separated by adding alcohol. When dried over sulphuric acid it weighed 5 grams and formed preparation D.3.1.

The solution filtered from the coagulum separating below 60°, when further heated, became turbid at 64° and yielded a flocculent coagulum at 70°. This second coagulum was filtered off after heating for some time at 88° and when washed and dried gave 3.5 grams of D.3.2. The filtrate from this gave no more coagulum on heating and after dialyzing free from chlorides was concentrated to small volume and precipitated by pouring into alcohol. This gave us 2 grams of D.3.3.

Seven grams of F.3 were dissolved in 280 cc. of 5 per cent. brine and the solution heated to 67°. The flocculent coagulum, which began to separate at 59°, was filtered out, washed and dried, giving 1.87 grams of F.3.1. The filtrate heated to 92° gave another coagulum which began to separate at about 70°. This, when dry, weighed 2.17 grams, F.3.2. The filtrate from F.3.2 dialyzed free from chlorides and, precipitated by alcohol, gave 1.39 grams of F.3.3.

Twenty-five grams of K.3 (the more soluble part of a precipitate obtained by adding an equal volume of saturated ammonium sulphate solution to a large quantity of egg white) were dissolved in about 800 cc. of 10 per cent. brine, the solution

TABLE II.—COMPOSITION, ETC., OF FRACTIONAL REPRECIPITATIONS OF THE INTERMEDIATE FRACTIONS OF TABLE I.

Prep.	Weight grams.	$[\alpha]_D$.	Ash free. C.	H.	N.	S.	P.	O.	Ash.	Coagulation temperature.	Remarks.
F.1.	7.50	36°4′	52.01	7.03	15.77D	1.768	0.069	23.353	0.36	T 58° F 64°	First coagulum small, above 70° more than in F.2.a.
F.2.	18.32	32°2′	52.36	6.95	15.59K 15.43K	1.689	0.117	23.374	0.47	T 68° F 68°	Only a trace below 70°; much above 70°.
F.2.a.	7.83	38°35′	51.54	6.94	15.19K 15.22K	1.920 1.900	0.081	24.319	0.60	T 60° F 63°	Considerable below 65°; above 70° more than in F.3.a.
F.3.	16.70	40°11′	51.32	6.72	15.34K	1.782 1.810	0.009	24.815	0.32	T 58° F 59°	Below 67°, nearly 35 per cent.; at 70° and above, nearly 40 per cent. coagulated.
F.3.a.	5.38	44°25′	51.29	6.77	15.30D	2.025	0.027	24.588	1.06	T 62° F 64°	More below 65° than in any of these fractions of F.

filtered clear and heated to 60°. The coagulum was washed with salt solution, suspended in water after passing through fine bolting-cloth to break up all lumps, washed thoroughly with water, and with alcohol, and was dried, giving 2.78 grams of K.3.1. Unlike ovalbumin, this coagulated proteid separated in a finely divided state, so that it could be easily washed through the cloth.

The filtrate from this coagulum was dialyzed free from chlorides and then evaporated to dryness below 60°, leaving a residue which weighed 17.2 grams and had a specific rotation of 37° 55′. This rotation being much greater than that of ovalbumin, the whole of this residue was dissolved in 10 per cent. brine, the solution heated in a boiling water-bath, the coagulum, K.3.2, filtered off, the filtrate dialyzed free from chloride, and the clear solution poured into alcohol. The substance, K.3.3, thus precipitated, weighed, dry, 1.39 grams, and consisted of ovomucoid as shown by its rotation, 61° 30′.

The preparation, K.3, was prepared from the precipitate produced by half saturating the egg white solution with ammonium sulphate, which precipitate is commonly supposed to consist almost wholly of globulin. Nevertheless our results show that K.3 contained about 11 per cent. of the albumin coagulating below 65°, 83 per cent. of ovalbumin, and over 5 per cent. of ovomucoid. That so much of this latter substance should be present in this preparation is surprising and shows the difficulty in separating ovomucoid from ovalbumin.

All these preparations were analyzed with the results given in Table III.

These figures show that an albumin coagulating at 55°–57° forms nearly 50 per cent. of the products obtained from D.3. This albumin contains somewhat less carbon, decidedly more nitrogen, and a little more sulphur than ovalbumin. Since it so closely resembles ovalbumin, and is so intimately associated with it the writer suggests that it be called *conalbumin*. The rest of the products from D.3 consists of about 35 per cent. ovalbumin and 18 per cent. ovomucoid. From F.3, 27 per cent. of conalbumin, 31 per cent. of ovalbumin, and 20 per cent. of ovomucoid were obtained.

This investigation of the protein constituents of the egg white

TABLE III.—COMPOSITION, ETC., OF PRODUCTS DERIVED FROM FRACTIONS D, F, AND K.

Preparation.	Weight, grams.	$[a]_D$.	Ash free. C.	H.	N.	S.	P.	O.	Ash.	Coagulation temperature.	Remarks.
D.3.1.	5.00	....	52.19	6.84	16.10K	1.67	0.010	23.19	0 85	T 48° F 52°	Formed about 48 per cent. of D.3.
D.3.2.	3.81	....	52.08	7.04	15.36	1.61	0.110	23.80	0.23	T 64° F 70°	Formed about 35 per cent. of D.3.
D.3.3.	2.00	61°10′	48.90	6.61	12.16K	2.34	none	29.99	2.33	....	No coagulum. Formed about 18 per cent. of D.3.
F.3.1.	1,87	....	....	....	16.03K 16.15K	17.3	trace	....	1.19	T 58° F 59°	Formed about 27 per cent. of F.3.
F.3.2.	2.17	....	....	....	15.16K	1.61	....	....	2.00	T 70° F 70°	Formed about 31 per cent. of F.3.
F.3.3.	1.39	61°20′	....	....	....	....	....	....	....	....	No coagulum. Formed about 20 per cent. of F.3.
K.3.1.	2.78	....	52.31	7.05	16.16K	1.71	trace	22.77	0.56	T 57° F 60°	Formed about 11 per cent. of K.3.
K.3.3.	1.39	61°30′	....	....	....	....	....	....	....	....	Formed about 5.5 per cent. of K.3.

shows them to be ovomucin, ovalbumin, conalbumin, and ovomucoid. These have the following properties:

Ovomucin is a glycoproteid recently discovered by Eichholz[1] to be present in small amount in egg white. This substance, precipitated by diluting egg white, has heretofore been regarded as globulin, but we find that nearly, if not quite, all of that which is so precipitated, is ovomucin.

From the whites of 240 eggs we obtained, although with some loss, 34.2 grams of ovomucin or about 7 per cent. of the proteid matter of the egg white, which is the proportion in which Dillner found the "globulin" to be present.

When freshly precipitated by dilution or dialysis, ovomucin loses its gummy character on thoroughly washing with water, but, when treated with salt solutions, forms a transparent gummy mass, which, on agitation, yields a clear but viscid solution.

When washed with alcohol and dried, ovomucin forms a light, white powder, partly soluble in sodium chloride brine, and gives a solution free from viscidity, which becomes turbid at 75°, and yields flocks at 78°. On boiling, this coagulum almost wholly dissolves and reappears on cooling.

Eichholz states that ovomucin dissolves in dilute sodium carbonate solutions. We find, however, that when treated with much 1 per cent. sodium carbonate, only an apparent solution results, for, when this is brought into filters, a clear, thin liquid passes through the paper from which, by adding acid, only an insignificant precipitate can be obtained, while a clear and very viscid liquid, containing almost all the ovomucin, remains upon the paper.

We have made, page 423, two preparations of ovomucin which had the following composition:

OVOMUCIN.

	A.1.a.	A.1.b.
Carbon	50.69	50.95
Hydrogen	6.71	6.85
Nitrogen	14.49	14.82
Sulphur	2.28	1.94
Oxygen	25.83	25.44
	100.00	100.00

[1] *Jour. of Physiology*, **23**, 167.

Ovalbumin is the chief constituent of egg white, 50 per cent. of its proteids having been obtained in this investigation in the form of perfectly crystallized preparations of this substance, while a large proportion of the remaining proteid matter also consisted of this albumin.

Heat Coagulum of Ovalbumin.—Solutions in pure water, containing 2.5 per cent. of pure ovalbumin, become turbid at 60°, and at 64° yield a flocculent coagulum.

When 10 per cent. of sodium chloride is added to these solutions the temperature of coagulation becomes higher, turbidity developing at 69°, and flocks at 70°.[1]

When its solution is evaporated to dryness below 50°, and the ovalbumin redissolved in water or in brine, a small amount of substance coagulating at the lower temperature appears to be formed. Thus, sodium chloride solutions containing 2.5 per cent. of B.1, B.2, or C.1, before these substances had been separated by evaporation at 50°, remained absolutely clear until heated to 68° or 69°, while similar solutions, made by dissolving the dried substance, became turbid at 59° and yielded flocks in small quantity at 63°.

Preparations of ovalbumin obtained by evaporating their solutions in pure water to dryness below 50°, do not completely redissolve when treated with water.

Solutions of some preparations thus made, become very rapidly turbid when filtered and gradually deposit a not inconsiderable quantity of insoluble matter. The amount of insoluble matter in all of our preparations was so small, that we have been unable to learn its nature, but in one case we obtained 0.72 gram from 15 grams of a preparation containing a larger proportion than any we had seen before. This insoluble substance, which contained 15.65 per cent. nitrogen, and 1.8 per cent. sulphur, was probably a mechanical coagulum, as it tended to separate at points of contact between the surface of the albumin solution and the vessel containing it. Sodium chloride added to the solution in sufficient quantity diminishes the amount, or prevents its formation.[2]

[1] Stärke (Pflüger's *Archiv.*, 12, 18) notes this effect of sodium chloride on the coagulation of the albumin.

[2] See Hopkins: *Jour. of Physiology*, 25, 324.

It has been stated by Hammarsten that with a constant amount of salt the temperature of coagulation changes with variable amounts of albumin in solution.

We, accordingly, prepared solutions of the dialyzed, but not dried, albumin which contained 5, 2.5, and 1.0 per cent. of ovalbumin and also similar solutions containing, in addition, 10 per cent. of sodium chloride. These, when heated very slowly in a large water-bath, coagulated as follows:

		Water.	10 per cent. salt solution.
5.0 per cent. albumin	turbidity	59°	68°
	flocks	64°	71°
2.5 " " "	turbidity	60°	69°
	flocks	64°	71°
1.0 " " "	turbidity	61°	69°
	flocks	67°	71°
0.5 " " "	turbidity	63°	..
	flocks	none even on boiling.	

These figures show no difference in temperature of coagulation, for solutions containing from 1 to 5 per cent. of ovalbumin, together with 10 per cent. of sodium chloride, but solutions of ovalbumin in pure water coagulate at a lower temperature than those containing 10 per cent. of this salt; those containing 5 and 2.5 per cent. of albumin, coagulate at one and the same temperature; those containing 1 per cent. coagulate somewhat higher, while a solution containing but 0.5 per cent. of albumin yields no flocks even on boiling.

When the proportion of albumin remains constant, while that of the salt increases, the temperature of coagulation rises, as the following table shows:

NaCl. Per cent.	Ovalbumin. Per cent.	
1.0	2.5	61° turbidity. 63° flocks.
3.0	2.5	63° turbidity. 65° flocks.
5.0	2.5	65° turbidity. 67° flocks.
10.0	2.5	68° turbidity. 70° flocks.

From these results we may state that solutions in water which contain from 2.5 to 5 per cent. of pure ovalbumin become turbid on heating to 60°, and yield a flocculent coagulum at 64°,

while those containing, in addition, 10 per cent. of sodium chloride become turbid at 68°, and flocculent at 70°.

In the preceding experiments no acid was added to the solution to be coagulated, the acidity of the ovalbumin (1 gram of albumin required 2 cc. of N/10 alkali for neutralization to phenolphthalein) being sufficient to bring about coagulation.

When the albumin was exactly neutralized to phenolphthalein, by adding potash, the solution remained clear when heated for some time at 100°. When this solution was treated with acid equivalent to the added alkali a large precipitate resulted which did not separate completely until a very slight excess of acid was added. Thus, when 0.5 gram of albumin was dissolved in 19 cc. of water, 1 cc. N/10 potassium hydroxide added, making the solution exactly neutral to phenolphthalein, and the mixture heated for ten minutes in boiling water, no coagulum was produced. When cooled, 1 cc. of N/10 hydrochloric acid was added, which gave a precipitate, from which a clear filtrate could not be obtained; with 1.2 cc., however, the precipitation was so complete that the filtrate gave no turbidity with alcohol. When 1 gram of albumin was dissolved in water and 2 cc. of N/10 ammonia solution were added, a quantity just sufficient to neutralize the acid reaction of the albumin, the solution remained clear after heating for some time at 100°. After cooling, on adding N/10 hydrochloric acid, the solution remained clear until nearly enough acid had been added to neutralize the 2 cc. of ammonia, but when the full 2 cc. were added the albumin was so completely precipitated that the solution filtered from the coagulum contained only a trace of protein matter. The solution, thus neutralized with either potash or ammonia, gave off hydrogen sulphide when heated and acidified.

To six tubes, each containing 0.5 gram of albumin, dissolved in 10 cc. of water, were added respectively 0.2, 0.4, 0.6, 0.8, 1, and 1.1 cc. N/10 hydrochloric acid, and then water enough to make each up to 20 cc. When these mixtures were heated in boiling water, the one with 0.8 cc. of acid yielded some coagulum, that with 1 cc. but a trace, while that with 1.1 cc. remained clear, showing 1 cc. of N/10 hydrochloric acid to be enough to convert 0.5 gram of albumin into a compound not coagulated by heat. The portions with 0.2 and 0.6 cc. were com-

pletely, while that with 0.8 cc. was incompletely, coagulated. When 0.5 gram of albumin was dissolved in 20 cc. of water, containing from 0.5 cc. to 3 cc. of N/10 acetic acid and heated in a boiling water-bath, the albumin was practically completely coagulated; with 5 cc. of acetic acid the solution became opalescent at 64°, and formed a firm, transparent jelly at 75°. On heating at 99° with 10 cc. of N/10 acetic acid the solution formed a clear, thin jelly.

When solutions in pure water of any of our purest preparations of ovalbumin were heated to boiling for some time the albumin was so incompletely coagulated that a clear filtrate could not be obtained.

Six 2-gram portions of pure ovalbumin, were dissolved, each in 100 cc. of water, and mixed with equal volumes of water, containing 1, 2, 3, 4, 5, and 6 cc. of N/10 acetic acid respectively, and the mixtures boiled and poured on filters. The portion with 1 cc. of acid clogged the filter, as the coagulum separated imperfectly, whereas the others yielded clear filtrates. These, when evaporated to dryness, left residues weighing 0.0492, 0.0332, 0.0320, 0.0446, and 0.0648 gram, respectively.

From this it is seen that with 1 cc. of N/10 acetic acid the coagulation is incomplete, while with 3 and 4 cc. the amount of matter remaining in solution is less than with 2 cc. or than with 5 and 6 cc. This dissolved matter, which is very soluble in water, yields a solution decidedly acid to phenolphthalein and to litmus, contains proteid, and reduces Fehling's solution. Since the proportion, in which it is produced, appears to depend upon the quantity of acid added, we are inclined to regard it chiefly as a product of the action of the added acid upon a small part of the albumin, whereby uncoagulable acid compounds are formed in small, but variable proportion.

Specific Rotation.—This was determined for solutions of the ovalbumin in pure water, with a Schmidt and Haensch polarimeter, the readings of the sugar scale being converted into degrees of circular polarization by multiplying by 0.346, and the amount of dissolved albumin ascertained by evaporating and drying the residue at 110°. Preparations B.1, B.2, C.1, and C.2 showed very nearly the same rotation as that found by us in our

former work for preparations of pure ovalbumin. These determinations gave the following values for $[\alpha]_D$.

A.1[1]	−29.31°	B.1	−29.53°
A.2[1]	−29.40°	B.2	−29.13°
H.1[1]	−28.60°	C.1	−30.03°
H.2[1]	−29.81°	C.2	−30.80°

The degree of rotation was found to be the same in sodium chloride solutions as in water, and also to be the same for the albumin in the dialyzed solutions of the crystals, as in solutions of the albumin which had been separated from such solutions by evaporation at 50°.

Since this paper was written we have received the number of the *Journal of Physiology* issued April 24, 1900, containing a paper on "Pure Albumin", by F. G. Hopkins, in which he gives the rotary power of pure albumin as −30.70°. The remarkable agreement between the rotation of the many fractional crystallizations, obtained by Hopkins, is much closer than between those observed by us, which is probably due to his superior polariscope and to the stronger solutions of the albumin, containing considerable amounts of ammonium sulphate, which he employed. Because solutions rich in pure albumin, when free from salts, are not easily obtained absolutely clear and tend to become turbid, probably from mechanical coagulation, we were generally not able to use solutions so rich in albumin as those employed by Hopkins. The difference of 1.3° between the values of $[a]_D$ found by Hopkins and the writer, is probably chiefly due to the different methods employed for determining albumin in the solution examined. We have shown, page 438, that when ovalbumin is coagulated in the presence of a minimum of acetic acid, about 1.5 per cent. of uncoagulated matter remains in solution. Whether this occurs under the conditions under which Hopkins coagulated his albumin requires further investigation. Hopkins admits that the method employed by us is the more accurate, assuming that all ammonium sulphate can be separated from the albumin solution. We believe that we have accomplished this in view of the great care we have taken to detect the presence of sulphates in our albumin preparations and feel quite sure that we have not overlooked a quantity of ammo-

[1] These are preparations of the old series formerly described, and the two first are *not* parts of fraction A of the present series.

nium sulphate sufficient to have caused a difference of 4 per cent. of the observed rotation.

Elementary Composition.—In the following table we give the analyses of our four purest preparations of ovalbumin, and also analyses of two preparations made by Hofmeister's method as described on page 441.

COMPOSITION OF OVALBUMIN.

	B.1.	B.2	C.1.	C.2.	No. 2.	No. 3.	Average.
Carbon.....	52.59	52.78	52.75	52.79	52.82	52.78	52.75
Hydrogen...	7.10	7.09	7.31	7.00	7.03	7.07	7.10
Nitrogen....	15.55	15.57	15.50	15.60	15.32	15.53	15.51
Sulphur.....	1.613	1.619	1.613	1.634	1.590	1.651	1.620
Phosphorus .	0.126	0.127	0.131	0.126	0.123	0.112	0.122
Oxygen	23.021	22.814	22.696	22.850	23.117	22.857	22.898
	100.000	100.000	100.000	100.000	100.000	100.000	100.000

ANALYTICAL METHODS.

Carbon and hydrogen were determined by using an open tube with copper oxide, lead chromate, and metallic copper, and finishing the combustion in oxygen.

Nitrogen was determined by Dumas' method, the air being removed by a Sprengel pump, the tube then filled with carbon dioxide set free by heating sodium bicarbonate at the front end, again emptied by the pump and this process repeated. In this way all the air was removed and no fixed gas was driven off on heating the reagents. Nitrogen was also determined by the Kjeldahl method and results in close accord with those of Dumas' method were obtained.

Sulphur was determined by fusing about 1 gram of the substance with sodium hydroxide and peroxide over an alcohol lamp. The reagents were proved to be free from sulphur.

Phosphorus was determined by fusing about 1 gram of the substance with sodium peroxide, dissolving the fusion in nitric acid, precipitating with molybdic solution and weighing as magnesium pyrophosphate.

The figures given in the above table agree well with those of others who have prepared and analyzed ovalbumin with especial care as may be seen in the following table:

COMPOSITION OF OVALBUMIN.

	C.	H.	N.	S.	P.	O.	
Hammarsten	52.25	6.90	15.25	1.64	...	23.69	uncoag.
Chittenden and Bolton....	52.33	6.98	15.89	1.83	...	22.97	"
" "	52.33	6.98	15.84	1.81	...	23.04	coag.
Bondzinski and Zoja.....	52.39	7.11	15.39	1.66	...	23.45	"
Osborne and Campbell....	52.75	7.10	15.51	1.62	0.12	22.90	
Hopkins.....	52.75	7.12	15.43	1.57			

There would be no longer any question about the composition of ovalbumin, were it not for Hofmeister's[1] analysis of a single preparation, and the recent assertion of Schulz,[2] that on crystallizing by the "acid process" a hydrate of Hofmeister's egg albumin is formed. This we have shown in our former paper to be untrue, for we there gave the results obtained by analyzing a preparation made exactly according to Hofmeister's method, which agreed quite well with the analyses of all our other preparations made by the acid method of Hopkins.

That there should be no question as to the composition of crystallized ovalbumin made according to Hofmeister's method we made preparation No. 2, recrystallized six times and coagulated by alcohol, No. 3, by recrystallizing four times, pressing the crystals on filter-paper, dissolving them in water, dialyzing the solution perfectly free from sulphate and evaporating the filtered solution at 50° to dryness.

Dried at 110° these preparations were found to have the following composition:

COMPOSITION OF OVALBUMIN CRYSTALLIZED BY HOFMEISTER'S METHOD.

	No. 2.	No. 3.	Average of the analyses given on page 365.
Carbon	52.82	52.78	52.75
Hydrogen	7.03	7.07	7.10
Nitrogen	15.32	15.53	15.51
Sulphur	1.590	1.651	1.62
Phosphorus	0.123	0.112	0.12
Oxygen	23.117	22.857	22.90
	100.000	100.000	100.000

These analyses are in almost exact agreement with the average given in the preceding table, and fail to confirm Hofmeister's figures for sulphur, or the conclusions drawn by him and by Schulz respecting the relation of the crystallized albumin to that heretofore obtained by other methods. The percentage of carbon found by us falls midway between that given by Hofmeister and the average of that given by the other investigators, with the exception of Hopkins. The very close agreement in composition between all the many fractions, having a constant rotatory power, analyzed by Hopkins and by ourselves, leaves

[1] *Ztschr. physiol. Chem.*, **16**, 187.
[2] *Ibid.*, **29**, 86.

little doubt as to the true elementary composition of crystallized ovalbumin, especially in view of the close agreement of these analyses with those of Hammarsten and of Bondzynski and Zoja.

In view of the statements recently made by Schulz concerning the proportion of sulphur in ovalbumin,[1] we used the very greatest care in determining this element. Our solutions of albumin were not only dialyzed until they gave no reaction with barium chloride, but until no sulphate could be detected in the water outside of the dialyzer, even after concentration. That our higher figures for sulphur are not due to ammonium sulphate, which could not be separated from the albumin by dialysis, is disproved, not only by the close agreement between our many analyses, but also by the fact that we obtained exactly the same figures from preparations free from conalbumin and ovomucoid, made by coagulating their dilute solutions at 70°–95°. Thus, we found in D.3.2, 1.61, in F. 2.1, 1.64, in F.3.2, 1.61, and in E.3.a, 1.63 per cent. We have no doubt that these figures very closely represent the true proportion of sulphur contained in the ovalbumin, especially as they agree with the figures which Hammarsten obtained,[2] by four different methods; namely, 1.67, 1.67, 1.62, and 1.58 and also with those of Bondzynski and Zoja. The results obtained by Hopkins,[3] which have come to our notice since this paper was written, leave no doubt whatever that the true proportion of sulphur is very close to 1.60 per cent., Hopkins finding an average of 1.57 per cent. while we find 1.62 per cent., the slight difference between our results being doubtless due to slight differences in our methods of operation.

It is to be noted that we have found a small but uniform quantity of *phosphorus* in all our preparations of crystallized albumin. In Table I, page 428, it is seen that the four crystallized preparations, B.1, B.2, C.1, and C.2, all contained 0.12 per cent. of phosphorus, whereas all the other non-crystalline preparations contained less, the amount diminishing as the proportion of ovalbumin diminished in the successive fractions. In Table

1 *Ztschr. physiol Chem.*, **25**, 16; **29**, 86.
2 *Ibid.*, **9**, 304.
3 *Jour. of Physiology*, **25**, 306.

III, page 432, it appears that D.3.1, consisting of conalbumin, contained but 0.01 per cent., whereas D.3.2, consisting of coagulated ovalbumin, contained 0.11 per cent., and D.3.3, consisting of ovomucoid, contained but a trace.

In our earlier series of preparations, this phosphorus was found in the ash, as calcium phosphate; in our present series, only a part of it was present in the ash, in which it occurred as sodium or potassium metaphosphate.

As our former series of preparations were dialyzed in river water containing calcium bicarbonate and our later series in distilled water, it seems highly probable that this phosphorus belongs to an acid united with the crystallized albumin in the same manner as the writer has shown that hydrochloric acid unites with edestin to form crystalline compounds.[1]

As to the nature of this phosphorized acid we have learned nothing, owing to the small proportion in which it is present.

THE CARBOHYDRATE SPLIT FROM THE OVALBUMIN BY BOILING WITH ACIDS.

These purest preparations of ovalbumin, which we have shown to have a constant specific rotation, the same composition and the same temperature of coagulation, when boiled with acids, all give solutions which yield considerable quantities of crystalline precipitates with phenylhydrazine.

When boiled with 3 per cent. hydrochloric acid for thirty minutes, the solution obtained reduces Fehling's solution, but fails to do so after boiling for three hours. The addition of Fehling's reagent to such solutions causes an intense biuret reaction, but no reduction of the copper salt takes place, even after adding considerable quantities of glucose. Evidently, by continued boiling, some substance is produced which prevents the reduction of the Fehling's solution. Blumenthal and Mayer[2] state that after long boiling this reduction is not so easily detected as after boiling for a short time.

Ten grams of each of the four preparations, B.1, B.2, C1, and C.2, were boiled with 200 cc. of 9 per cent. sulphuric acid for three hours and the solutions neutralized to litmus with baryta. The soluble matter was filtered out, washed with water, and the filtrate

1 This Journal, **21**, 486.

2 *Ber. d. chem. Ges.*, **32**, 274.

and washings were evaporated to a volume of 100 cc. To this solution 10 grams of sodium acetate were added and 5 cc. of a mixture of equal volumes of phenylhydrazine and 99 per cent. acetic acid, and the whole heated for three hours in boiling water. After standing over night, the crystalline precipitates were filtered off and washed rapidly with water and then with absolute alcohol, which removed a considerable quantity of deep red-colored, amorphous substance. The washed precipitates were then dried at 110° and weighed.

In order to learn the effect of the presence of ovomucoid, upon this precipitation with phenylhydrazine, 10 grams of G.4, which had a specific rotation of 39° 22′, and contained much ovomucoid, were treated in the same manner as these albumin preparations and at the same time with them.

For comparison, 100 cc. of a solution containing 1 gram of glucose were treated with phenylhydrazine under the same conditions. The filtrate from each phenylhydrazine precipitate was further treated with 5 cc. of phenylhydrazine and 10 grams of sodium acetate and a second precipitate obtained. From the filtrates from the second precipitations no more could be separated by further additions of phenylhydrazine. The weight of each precipitate dried at 110°, is given in the following table:

	B.1.	B.2.	C.1.	C.2.	G.4.	Glucose.
	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.
1st	0.2619	0.1327	0.1831	0.0902	0.2175	0.3098
2nd ...	0.1030	0.1027	0.0980	0.0281	0.2684	0.3187
	0.3049	0.2354	0.2711	0.1183	0.4859	0.6285

Since from C.2 we got so much less of this substance than from the others, we repeated this experiment, using for the first precipitation 10 cc. of the phenylhydrazine-acetic acid mixture and 20 grams of sodium acetate. In this way we got

C.2.

	Gram.
1st	0.1787
2nd.....	0.0894
	0.3681

The crystals of the osazone obtained from the albumin consisted of feathery sprays and always appeared distinctly differ-

ent from the aggregates of needles yielded by glucose. Two different preparations of the phenylhydrazine compound were separately recrystallized by dissolving in boiling absolute alcohol, addin gwater, boiling until most of the alcohol had been expelled, and then allowing the solution to cool slowly until the substance separated in beautiful, yellow crystals of the same form as the original precipitate. These melted at 187° and 189°.

The amount of this osazone indicates that a considerable quantity of carbohydrate is split from our albumin preparations by boiling with acids. The quantity of osazone which we obtained from our preparations varied from one-third to one-half of that given by 1 gram of glucose under like conditions, and if the compound from the albumin is precipitated in the same proportion as the glucosazone we may infer that the albumin yields on hydrolysis from 3 to 5 per cent. of carbohydrate. The amount of osazone which we have actually obtained corresponds to from 2–2.5 per cent. of carbohydrate calculated as glucose. Hofmeister[1] obtained from 1 gram of ovalbumin 0.13 gram of osazone, from which he infers the presence of 15 per cent. of carbohydrate.

Does this carbohydrate come from the ovalbumin or from a residue of ovomucoid which we have not separated by our fractional crystallizations? Seemann[2] obtained a copper oxide reduction from ovomucoid corresponding to 34.9 per cent. of glucose. If this proportion is correct, we must have at least 6 per cent. of ovomucoid in our albumin preparations, to account for the minimum yield of osazone, corresponding to 2 per cent. of carbohydrate. This is probably the least quantity of ovomucoid which could yield this quantity of osazone. From the incompleteness of precipitation of osazone, it is probable that the quantity of ovomucoid would have to be more than twice as great to yield the quantity of osazone found. From G.4, which had a specific rotation of 39° 22′, we obtained nearly twice as much osazone as from the ovalbumin, due unquestionably to the presence of ovomucoid in this preparation. If this increased yield of osazone is attended by an increase of 10° in the rotation, an admixture of ovomucoid sufficient to yield such quantities of

[1] *Ztschr. physiol. Chem.*, **24**, 170.

[2] *Archiv. f. Verdaungskrankheit*, **4**, 275.

osazone would manifest itself by variations in the rotation of these albumin preparations, it being improbable that so much ovomucoid was admixed in uniform proportion in the successive crystallized precipitates. We have shown on page 438 that the preparations of ovalbumin when coagulated by heat, left in solution a small quantity of uncoagulable matter amounting to about 1.6 per cent. of the albumin, the nature of which we have been unable to discover, owing to its small quantity. It may be ovomucoid, since it contains proteid matter and yields a copper-reducing body on boiling with acids, but in general appearance and manner of dissolving it does not behave like ovomucoid. As already stated on page 439, we think it more probable that this substance is a product of the action of acetic acid, whereby a small quantity of uncoagulable acid compounds of the albumin is formed. Hydrochloric acid, added in the same proportion as acetic acid, converts, in the absence of soluble salts, nearly all of the albumin into such uncoagulable compounds. A somewhat greater quantity of acetic acid does the same, entirely preventing coagulation. We therefore feel almost certain that the carbohydrate does not originate in admixed ovomucoid, but is derived from the substance constituting the crystallized ovalbumin.

This belief is strengthened by the fact that, so far as we know, all but two of the several investigators who have thus examined coagulated egg albumin, have found evidence of the presence of considerable quantities of carbohydrates among its hydrolytic decomposition products, although most of them have endeavored to separate every trace of ovomucoid. Spencer,[1] who failed to find carbohydrate, boiled his coagulated albumin for several hours with weak potash and then for thirty minutes with 10 per cent. potash, a procedure which might lead to the destruction of carbohydrate. Moerner[2] who also found no carbohydrate, gives no account of the method he used in preparing the ovalbumin.

Hammarsten regards crystallized ovalbumin as a glycoproteid and considers the carbohydrate group to be a constituent of a non-proteid substance combined with the protein substance proper.

1 *Ztschr. physiol. Chem.*, 24, 354.
2 *Centrbl. Physiol.*, 7.

This is certainly true of the nucleoproteid of the pancreas from which Bang obtained guanylic acid, which on hydrolysis yielded about 30 per cent. of carbohydrate. The mucins also appear to be compounds of protein matter with sugar-yielding substances.

We have stated our belief that crystallized ovalbumin is a compound of some acid with protein substance and consider it quite probable that this acid contains the carbohydrate group.

We have been unable to obtain any evidence of carbohydrate in edestin, the crystallized globulin of hemp-seed, nor, according to Hammarsten, can a carbohydrate be split from casein, vitellin, myosin, and fibrinogen. This subject requires further careful study before a definite conclusion can be reached. At present the preponderance of evidence indicates that the carbohydrate is not derived from the protein molecule, but from substances combined with the protein as it is obtained from the tissues or secretions.

Conalbumin.—Our fractions of the proteids of egg white obtained after separating the crystallized fractions, all have a higher rotation and sulphur content and a lower temperature of coagulation than ovalbumin, as is shown in Table I. In Table III we showed that these fractions could be separated into two products by heating their solutions to 65°, filtering off the coagulum, and then heating the filtrate to 90°. In the filtrate from this latter coagulum a substance remained which was not coagulated by boiling.

The body coagulating at the lower temperature we designate *conalbumin*, on account of its close relation in properties and composition to ovalbumin. What this relation may be, we have not determined. Conalbumin and ovalbumin may be different compounds of the same protein, or the former may be a derivative of the latter involving a molecular change.

COMPOSITION OF COAGULATED CONALBUMIN.

	D.3.1.		F.3.1.		K.3.1.		Average.
	I.	II.	I.	II.	I.	II.	
Carbon	52.17	52.20	....	....	52.47	52.14	52.25
Hydrogen ...	6.98	6.70	....	....	7.02	7.09	6.99
Nitrogen	16.04	16.16	16.03	16.15	16.16	....	16.11
Sulphur	1.67	....	1.73	....	1.71	....	1.70
Oxygen.....	23.14	....	....	...	22.64	....	22.95
	100.00				100.00		100.00

These figures show very little difference in composition between ovalbumin and conalbumin, the carbon being about 0.5 per cent. lower, the nitrogen about 0.53 per cent. and the sulphur 0.08 per cent. higher.

It is possible that the higher sulphur is due to a little ovomucoid carried down with the coagulum, but this is hardly probable, as the ovalbumin coagulated in the filtrates from these preparations contained 1.61 per cent. of sulphur, which is exactly the quantity found in the most thoroughly purified preparations of ovalbumin.

Temperature of Coagulation.—When a solution of conalbumin is heated sufficiently, a finely divided flocculent coagulum separates which is very different from the dense masses that form in a similar solution of ovalbumin. As we could not separate the conalbumin from the other associated proteids except by coagulation, the temperature of coagulation of this albumin could not be determined under definite conditions. The temperature at which this substance begins to coagulate is influenced by the proportion of salts present. From a solution containing 10 per cent. of sodium chloride it separates at a lower temperature than from solutions in pure water. From the former solutions it apparently separated completely below 60°, whereas from the latter it is impossible to separate it from the ovalbumin because the latter begins to coagulate before the separation of the conalbumin is complete.

From solutions containing 10 per cent. of sodium chloride the preparations D.3.1 and K.3.1 separated below 60°, the solutions becoming turbid at about 55°. When 2.5 per cent. of K.3, D.3, and F.3 were each dissolved in 10 per cent. brine their solutions became turbid at 57°, 52°, and 58°, and flocks separated at 58°, 55°, and 59°, respectively.

Specific Rotation.—This we were unable to determine directly but indirectly the following results were obtained: of F.1, 1.5 grams were dissolved in 10 per cent. brine, the solution heated to 65°, and the coagulum filtered out and washed. The filtrate was heated to 98° and the coagulum also filtered out and washed. The nitrogen was then determined in each coagulum and in the final filtrate, and the proportion of conalbumin, calculated on the dry preparation, was found to be 25 per cent; of ovalbumin

62.7 per cent; and of ovomucoid 12.2 per cent. Subtracting the amount of rotation due to the sums of the two latter from the total rotation of F.1, we found the amount of rotation caused by the 25 per cent. of conalbumin, which for 100 per cent. was equal to $[\alpha]_D$—39°.

In the same way we found F.3 to contain 36.6 per cent. of conalbumin, 39.7 per cent. of ovalbumin, and 23.7 per cent. of ovomucoid, from which we calculated for the conalbumin $[\alpha]_D$—36°. F.2.a contained 30.9 per cent. conalbumin, 48.9 per cent. ovalbumin, and 20.4 per cent. ovomucoid, so that in this preparation we found $[\alpha]_D$ equal to —36° 21′.

Of course such determinations cannot be accepted as final but they are sufficiently accurate to show that conalbumin has a different specific rotation from ovalbumin and is therefore a different substance. This conalbumin appears to be identical with albumin II, described by Panormoff,[1] but so far as we can infer from this abstract of his original paper[2] the substance which he describes under this designation corresponds very closely with our fractions C.3, D.1, D.2, and D.3, whose mean rotation and other properties are the same as those given for albumin II.

These fractions we have shown to be mixtures of ovalbumin, conalbumin, and ovomucoid.

Ovomucoid.—After separating all the proteids coagulable by heat, Neumeister[3] found in the white of eggs a substance which he called pseudopeptone. Later Mörner[4] showed this to be a glycoproteid and named it ovomucoid, under which name it is now generally known.

In Table I it is to be noticed that all the fractions following those of the crystallized ovalbumin show an increasing value for $[\alpha]_D$ and proportional thereto an increasingly greater content of sulphur. This is chiefly due to ovomucoid associated with these fractions. In connection with the detection of sugar in the albumin molecule, it is important to recognize the difficulty with which ovomucoid can be separated from ovalbumin by fractional precipitation with ammonium sulphate.

As we have just shown, fraction F.1 contained 12.2 per cent.,

[1] *Chem. Centrbl.*, **11**, 487 (1898).
[2] *Jour. russ. phys. chem. Ges.*, **30**, 302.
[3] *Ztschr. Biol., N. F.*, **9**, 369 (1890).
[4] *Ztschr. physiol. Chem.*, **18**, 525 (1893).

F.3, 23.7 per cent., and F.2.a, 20.4 per cent. of ovomucoid. These fractions were obtained from those immediately following the separation of the crystalline ovalbumin, and F.1 was precipitated by simply adding an equal volume of saturated ammonium sulphate solution to the solution of these fractions.

We have obtained two preparations of ovomucoid in sufficient quantity for analysis and have found them, when dried at 110°, to have the following composition, which agrees with that given by Zanetti[1] and by Mörner[2].

COMPOSITION OF OVOMUCOID.

	E.4	D.3.3.	Zanetti.[1]		Morner.[2]
Carbon	49.02	48.90	48.94	48.75	....
Hydrogen	6.45	6.61	6.94	6.90	....
Nitrogen	12.71	22.16	12.46	....	12.65
Sulphur	2.38	2.34	2.22	....	2.20
Oxygen.....	29.44	29.99	29.44	....	....
	100.00	100.00	100.00		

The specific rotation was found to be E.4, —61° 38′; D.3.3, —61° 10′; F.3.3, —61° 20′; K.3.3, —61° 30′.

NOTE.

The Employment of Ammonium Molybdate as a Test for Tin. —In the April number of this Journal there is a note by Mr. Allen Rogers on the use of ammonium molybdate as "a very delicate test for tin." Mr. Rogers does not refer to my paper on this subject in the *Chemical News* for December 15 last,[3] and I shall therefore assume that he has not seen it. Perhaps I may therefore be permitted to state briefly the substance of that paper.

Although the color reaction involved is a well-known test for molybdenum, I have been unable to find that, prior to the appearance of my paper, it had ever been suggested as a test for tin; and, having regard to its extreme delicacy in this respect, it seems highly improbable if it had been, that the fact would have been allowed to go into oblivion. My own experience, and I

[1] *Ann. di Chim. e Farmac.*, No. 12, *1897*.
[2] *Zeit. physiol. Chem.*, **18**, 525.
[3] *Chem. News*, **79**, 282 (1899).

believe that of others also, is that the detection of tin in complex mixtures is, with the methods of the text-books, a great difficulty to students, whereas the test now proposed makes it exceedingly easy—assuming ordinary care to be taken.

For the success of this method, two important points must be attended to: *First*, in reducing stannic chloride to stannous chloride, the zinc employed must be entirely free from traces of tin. Samples of commercial zinc that I tried were found quite useless for the purpose. I employed pure zinc supplied by Harrington's for my experiments. *Second*, since a very dilute stannous solution, on exposure to air, becomes oxidized to stannic in a marvelously short time, as soon as the zinc and tin are completely dissolved in hydrochloric acid and the solution is diluted, the ammonium molybdate must be added at once.

I observe that Mr. Rogers finds that "a solution of stannic chloride containing 0.0000042 gram to 1 cc. gives a faint blue color with ammonium molybdate." This corresponds to 1 part of stannous chloride in rather less than 240,000 parts of solution. Had he used boiled water in making up his solution, he would have found that the delicacy of the test is far greater than this. By taking the precautions described in my paper, I was enabled to get a delicacy of 1 in 1,500,000. I concluded, however, that under ordinary conditions of working, a very distinct blue color is given by the presence of 1 part of stannous chloride in anything up to 100,000 parts of solution.

I am very glad to find that Mr. Rogers confirms my experience in three points: (1) that ammonium molybdate is a much better reagent than mercuric chloride for stannous chloride; (2) that the reagent must not be added without first of all diluting the hydrochloric acid present in the solution to be examined; and (3) that the ordinary laboratory reagent gives results that are most satisfactory.

I shall merely add that the method has been used in this laboratory during the past five months with uniform success.

J. P. LONGSTAFF.

CHEMICAL LABORATORY, UNIVERSITY OF EDINBURGH, May 22, 1900.

NEW BOOKS.

RÉPERTOIRE GÉNÉRAL OU DICTIONNAIRE METHODIQUE DE BIBLIOGRAPHIE DES INDUSTRIES TINCTORIALES ET DES INDUSTRIES ANNEXES DEPUIS LES ORIGINES IUSQU' A LA FIN DE L'ANNEE, 1896. Technologie et Chimie. Par Jules Garçon. Tome I. Paris: Gauthier-Villars. 1900. 74 pp. Premier Fascicule.

This dictionary of the bibliography of the dyeing industries and allied subjects, is one of the most important pieces of bibliographical work that has ever been attempted for the use and convenience of chemists and technologists. It originated with the chemical section of the Société Industrielle de Mulhouse in 1892, and, after a labor of five years, the finished work was submitted to the society in 1897, when, after careful examination at the hands of a commission of experts, it was awarded the Daniel Dollfus grand prize of 5,000 francs and a medal of honor. The magnitude of this piece of bibliography will be understood when we state that the author makes reference to some 2,000 distinct works and to 110 periodicals, which latter make up about 5,000 volumes more. The first part of Vol. I, at present under notice, is devoted to the introduction, outlining the scope of the work and giving a list of the bibliographic sources availed of, both official publications and records and general and special bibliographical publications and catalogues; an alphabetical list of subject-headings under which the material is arranged; and a list of the periodicals from which the references are taken. The second part of Vol. I will be devoted to the indices of authors cited and subjects treated, while Vols. II and III will be the reference dictionary proper arranged under the various subject-headings. The scheme seems, on examination, to be a very complete one and the part of the work now in hand promises well for the way in which it will be executed.

The first part of Volume I has been issued, as just noticed, and the second part of the volume is stated to be now in press; Volume II is ready for distribution and Volume III is promised by the end of 1900.

S. P. SADTLER.